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# Canadian Journal of Zoology

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## A METHOD FOR CONTINUOUS DETERMINATION OF DISPLACEMENT ACTIVITY IN A GROUP OF FLYING INSECTS[1]


D. K. EDWARDS[2]



### Abstract

A method is described for obtaining a continuous record of the amount of flight activity occurring in a group of flying insects. The test insects are kept in an earthed metal cage. When an insect flies it accumulates a positive charge on its body. This charge influences a sensitive electrometer-probe in the center of the cage. The impulse resulting from the flight is amplified by the electrometer and is recorded as a deflection on a pen-recorder chart. By counting the number of deflections in a given time an activity curve for the test insects can be plotted.


### Introduction

Numerous methods have been developed for obtaining estimates of the amount of activity exhibited by an insect in a given time. Cloudsley-Thompson (1) has summarized many of these. Some attempts have been made recently to reduce, at least partially, the technical problems encountered as a result of the small size of an insect (2). In addition, some photographic techniques have been developed through which it is possible to estimate the amount of movement occurring in a group of insects in a given period of time (3, 4). The photographic methods are improvements upon some of the older ones in that (*a*) they are independent of the mass of the organism being tested, and (*b*) more than one insect can be used at once. All of the methods described to date are useful in specific experimental situations, but their usefulness under other conditions is sometimes limited.

In some recent experiments it became necessary to monitor the activity of a group of flying insects. However, the nature of the experiments precluded the use of a camera for photographic measurements of activity. Further, since the activity effects under study were of relatively short duration, it was desirable to obtain a continuous activity record, instead of a series of intermittent estimates. It was necessary for the experiments to have the group of insects confined in a cubical cage, with sufficient room for flight to occur. The technique which solved these problems is based upon electrical influence of a flying insect upon a highly sensitive probe within the insect cage. Sudden changes in potential on the probe as the insects fly past it are continuously recorded.


[1]Manuscript received August 4, 1960.
Contribution No. 681, Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada.


[2]Forest Biology Laboratory, Victoria, B.C.





[The previous number of Can. J. Zoology (38, 839–1020) was issued October 20, 1960.]

## Method

A schematic diagram of the apparatus is given in Fig. 1. The test container was a steel box, 9 inches cubed (*a*). Two opposite sides of the box were cut out and fitted with wire window-screen, and a hole 1 in. in diameter was cut in the center of the solid top. Projecting into the box, to a point somewhat short of its center, a metal sleeve (*b*) was soldered around the circumference of the hole. The box was put inside a steel cabinet with 17-in. sides (*c*), and oriented with one screened side facing outward and the hole upward. A 1-in. hole in the top of the cabinet was aligned with the hole in the insect box. Both cabinet and box were earthed.

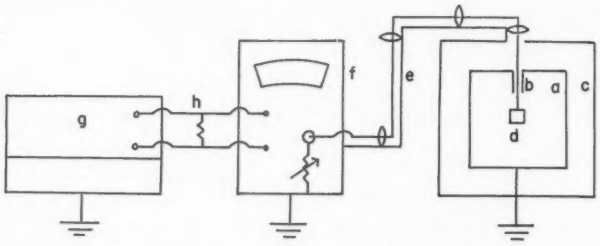


FIG. 1. Diagram of flight-activity apparatus.

The static-charge sensitive element within the insect cage consisted of a standard electrometer-probe (*d*) on the end of a Keithley model 6101 shielded test-lead (*e*). The probe is essentially the exposed tip of the shielded test-lead, and is highly insulated from earth. A piece of copper sheet, 2 cm square, was fastened to the end of the probe to increase its sensitivity. The probe, connected to the end of its shielded lead, was passed through the holes in the two boxes, through the metal sleeve, and fixed with its sensitive end approximately at the center of the insect cage.

The shielded test-lead from the probe was connected to a Keithley model 600 electrometer (*f*) with an output suitable to drive a pen recorder. A Varian G-10 recorder was used (g), with the span set at 100 mv. Since the electrometer gave a maximum output of 1 volt, it was necessary to reduce this voltage with a shunt across the output (*h*).

Approximately 24 hours prior to the experiment the test insects (usually 10–12 adult blowflies) were introduced into the insect cage without etherization. Within the cage was a water supply for the insects, and sugar crystals could be added when desired. The insect cage was placed in position within the larger steel cabinet. The electrometer probe was pushed into the cage through the holes in the top. The instrument cases and both boxes were connected to earth. A constant light source was fixed above and in front of the cage.

A few hours prior to the experiment the electrometer and the recorder power supply were switched on for warm-up. The shunt between the high side of the electrometer and earth was set to an optimal value to prevent accumulation of charge on the probe (usually $10^9$ ohms) and the voltage range

adjusted (usual ranges were 0–0.03 v, and 0–0.01 v). The chart speed was set to run at 2 inches per hour. The experiment was begun by releasing the shorting button of the electrometer and switching on the chart drive and servo-unit of the recorder. Changes in potential of a few millivolts on the probe would now be recorded as deflections on the chart.

## Results and Discussion

When there was little or no activity in the insect cage, the recorder pen drew a fairly steady base line. While the insects rested upon the earthed metal cage they could not accumulate a net charge. However, immediately an insect flew to another spot within the cage a positive charge accumulated upon its body. The charge was picked up by the electrometer probe and the pen recorder showed a sudden, positive deflection at right angles to the base line. Thus, by counting the number of deflections in a given time, an estimate of the amount of flying activity occurring within the cage was obtained. The length of each deflection is determined by the proximity of the flight to the probe. Thus, some information may be obtained regarding the distance of the flying insect away from the take-off surface.

A portion of a record obtained with this technique is shown in Fig. 2. The slight angle of the tracing is the result of electrometer drift; the record becomes straighter after several hours continuous running. For the record five male and five female adult blowflies (*Calliphora vicina* R.D.) were put into the insect cage 1 week prior to the experiment. They were kept under constant illumination and were fed daily with a sucrose solution. The activity record was run for 5 hours. The illumination was interrupted with three 15-minute periods of total darkness (D). The trace shows that flight activity ceased completely during each period of darkness. When light was restored flight activity resumed. During the experiment, temperature (22° C), relative

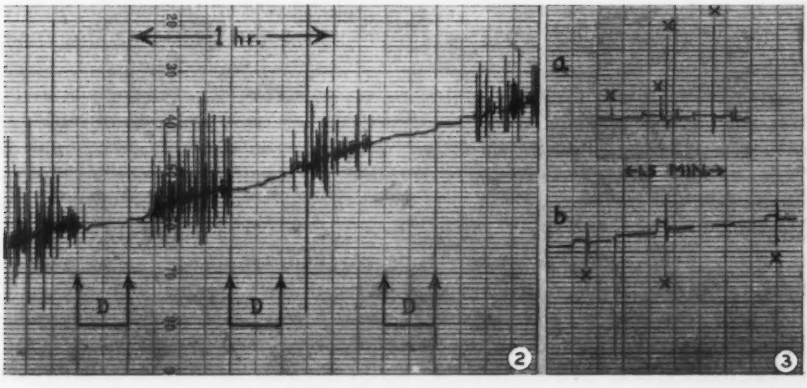


FIG. 2. Chart record of *Calliphora* flight activity in light interrupted by 15-minute periods of darkness (D); positive deflections above base line, negative below.

FIG. 3. Chart records of (*a*) *Calliphora* flights, and (*b*) "dummy" insect "flights"; positive deflections above base line, negative below; initial deflection indicated by ×.

humidity (40%) and barometric pressure (1018 mb) were constant. The insects were isolated from external disturbances during the experiment; the observer was in the same room with the apparatus only when the light was switched off or on.

The deflection on the pen-recorder chart resulting from an insect flight is due to accumulation of a positive charge on the flying insect, not merely to a capacitance effect between insect and probe. The evidence for this is twofold. First, a pure capacitance effect was obtained by allowing a "dummy" insect made of aluminum foil to drop from an earthed position at the ceiling of the insect cage, past the probe, on to the floor. The deflections caused by the "dummy" on the recorder chart were compared with deflections produced in a typical insect experiment. In Fig. 3, deflections caused by flying insects are shown in (*a*): the time between each chart division is 22.5 seconds. Part (*b*) shows deflections produced by the falling "dummy" insect. In each case the direction of *first* deflection is indicated by ×. Whereas the first deflection caused by an insect flight was in a positive direction, the first deflection with the "dummy" was negative. The insect, therefore, apparently generated the positive charge on itself after it left its earthed resting position. The slight "bump" just prior to each deflection in (*b*) was caused by introduction of the end of a thin glass rod into the cage to dislodge the "dummy" insect from its position.

Occasional landings of an insect on the probe furnished the second point of evidence showing the charge on a flying insect. These caused the recorder pen to go off scale in the positive direction.

It has usually been desirable to convert the pen-recorder tracing into an activity graph of plotted points. First, an arbitrary "threshold" spike height was set, for clarity in resolving the deflections. The number of spikes exceeding this limit were then counted in each chart division (7.5 minutes). The threshold spike height chosen does not appreciably affect the trends of the plotted curves, except that too high a threshold (e.g. 1.5 cm.) increases the number of zero values. When the values obtained are plotted against time a good graphic representation of the changes in spike density with time is obtained.

An additional type of behavior change is sometimes evident in these activity traces. Increased activity in a group of insects may be in the form of walking, rather than flying. This may be shown by the trace as an increased amount of "wiggle" in the base line. In traces of this type the sudden deflections due to flights may not be numerous; instead, the base line of the trace follows a very erratic course with increased walking activity. This is undoubtedly a capacitance effect occurring between the probe and the bodies of the insects moving upon the earthed sides of the cage. It is difficult, if not impossible, to translate this type of record into numerical data which can be plotted against time. However, in some cases, this transformation may not be necessary.

The method described in this report would probably prove useful in studying swarming activity in certain Diptera. Further examples of results obtained with this method will be found in subsequent papers dealing with some environmental effects on insect activity.

## Acknowledgments

The author gratefully acknowledges the assistance given by Dr. C. D. Maunsell and others at the Pacific Naval Laboratory, Esquimalt, B.C., in making available some of their apparatus during the first part of these studies.

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# THE COURSE OF BLOOD CIRCULATION IN MATURE EMBRYOS OF THE COCKROACH BLABERUS GIGANTEUS (L.) (ORTHOPTERA:BLATTIDAE)[1]

J. W. ARNOLD

## Abstract

In mature embryos of *B. giganteus* the blood circulated vigorously through definite and often intricate pathways. Although the course through major sinuses depended upon the structure and location of organs and muscles and on the propulsive force of the heart, the direction of flow in the appendages and through the narrow spaces among the tissues was governed also by the aspirative force of adjacent major currents. The course of circulation is described in detail for each region of the body with special emphasis on that in the head. The structure and action of the frontal pulsatile organs is also described.

## Introduction

The general course of blood circulation in insects is well known and is described particularly in textbooks of insect physiology, e.g. Roeder (7), and Wigglesworth (9). The details of circulation are less well known, especially for species and for regions of the insect body where direct observation of the flowing blood is precluded by factors such as the obscure nature of the cuticle, the presence of opaque organs and muscles, or the excitability of the insect. The view is generally held that due to the absence of confining vessels the blood circulates rather slowly and haphazardly in most regions of the body and is actively directed only in the heart and in localized regions where accessory pulsatile organs occur.

Recently, while studying the haemocytes in living embryos of the giant cockroach, *Blaberus giganteus* (L.), the writer was impressed by the intricacy of the circulatory routes and the vigor of the circulation in the insect at this stage of development. The blood coursed rapidly and in definite directions through a network of sinuses in the head and continued to flow in fairly well-defined streams in other regions of the body. This paper describes the circulation in embryos that were almost fully developed and discusses some general aspects of open blood circulation. Although changes in the circulatory routes undoubtedly occur later in relation to wing development and the increasing volume of muscles and other tissues, the description presumably also applies in general to the postembryonic stages of this and similar species.

## Materials and Methods

*B. giganteus* is an ovoviviparous species and the female normally carries the oötheca in an internal brood sac until eclosion. However, by using flat forceps the oötheca could be withdrawn easily and safely from the carbon-dioxide-anaesthetized female, especially when the embryos were nearly mature.

[1]Manuscript received July 9, 1960.
Contribution from the Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

The thin, cuticular oöthecal membrane was removed and the embryos, which were approximately one centimeter long, were placed separately on filter paper that was kept moist with physiological saline solution in small, covered crystallizing dishes. They were held at 25° C, the rearing temperature for the roaches. The cuticle was transparent and the organs and muscles were differentiated but not yet entirely opaque. The circulatory system was functioning vigorously.

The immediate preparation for observing blood circulation involved removal of the chorion with fine forceps and immersion of the embryo in physiological saline solution in a deep, hollow-ground microscope slide. The embryo was caused to sink by adding a trace of detergent (Tween 20: Atlas Powder Co., Brantford, Canada) to the solution. In this medium and in the light of the microscope lamp blood circulation in the embryo continued in normal fashion for periods of about 30 minutes. Thereafter the rate of flow decreased noticeably and the frontal pulsatile organ functioned sporadically. Recovery was rapid on removal from the solution and some embryos that were especially transparent were used for more than one period of observation. Each observation period was terminated when an abnormal condition was detected. The observations were made at low magnifications (80× and 120×) with a Leitz Ortholux microscope using bright-field illumination with a Wratten B (green) filter. The morphology of the frontal pulsatile organ was studied by direct observation of the organ in vivo, by dissection of the heads of several nymphs and adults, and from sections of the heads of embryos and nymphs prepared by standard histological methods.

## Observations and Discussion

The general course of blood circulation in the embryo was similar to the schematic system for insects described by R. L. Beard in Roeder's textbook (7) and by others and is illustrated here (Fig. 1). However, although the blood percolated randomly among the organs and tissues throughout this circuit the main streams were more intricate and definable than is implied in the scheme or by the designation of an "open circulatory system". Furthermore the blood flow along these routes was remarkably vigorous. This was especially true for the head and thorax where the pressure was relatively high due to the proximity of these regions to the heart outflow and to the confinement of the blood there by the concentration of organs and tissues. It was less true for the abdomen where the pressure was dissipated in the large haemocoel and also for the appendages which were located off the main blood stream and offered some resistance to free circulation.

Circulation was maintained principally by the propulsive and aspirative forces of the heart, with some assistance from involuntary organ movements and, in the antennae, from accessory pulsatile organs. Pulsations that corresponded to those at the heart-outflow in the head were detectable as far away as the perineural sinus in the thorax. Beyond this point the pulsations were not discernible, and in the perivisceral regions the rate of flow slowed

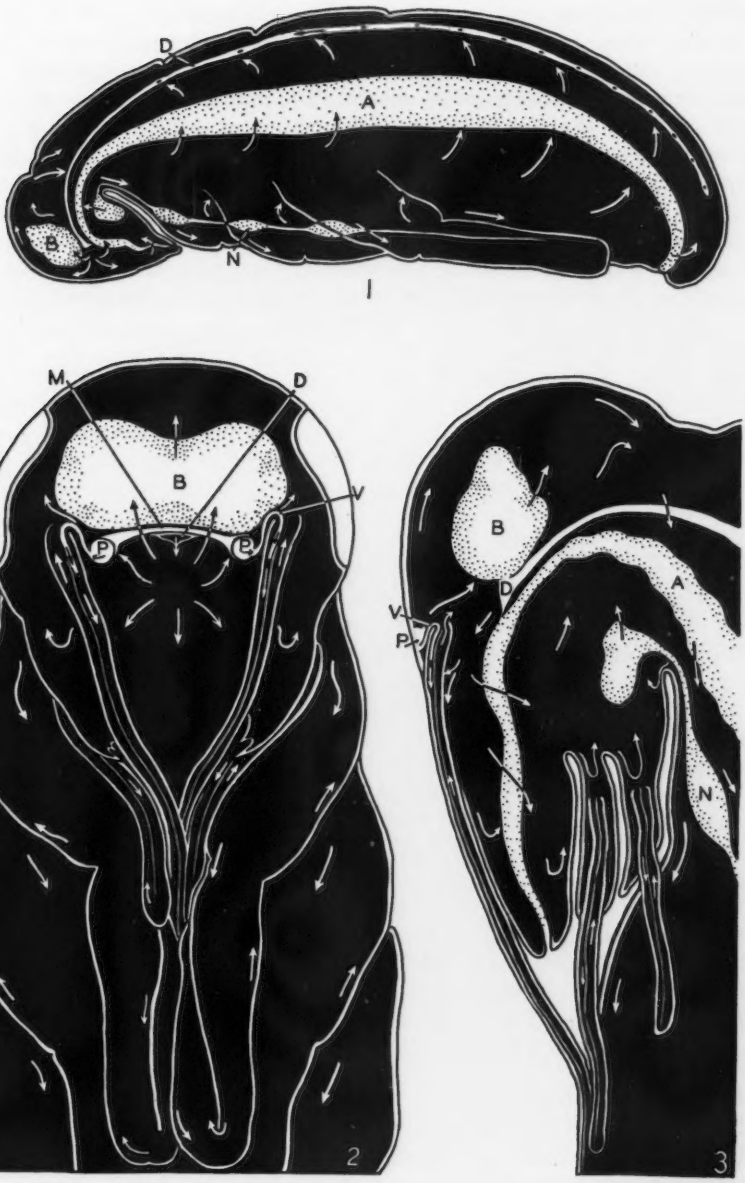



FIG. 1. Lateral view of the embryo (diagrammatic) showing the general course of blood circulation. FIGS. 2 and 3. Frontal and lateral views of the head region (diagrammatic) showing details of circulatory routes.

ABBREVIATIONS: A, alimentary canal; B, brain; D, dorsal vessel; M, connecting muscle bundle; N, ventral nerve cord; P, frontal pulsatile organ; V, antennal blood vessel.

until the blood approached the pericardial sinus and came within the aspirative field of the heart. The course of the major streams, which occupied the large sinuses of the body, was mainly unobstructed and obviously related to the position of organs and groups of muscles. The course of the minor streams, which occupied the remaining available spaces, seemed to depend on the position of tissues and to be controlled by the aspirative force of the major streams. Thus blood was continually propelled into the narrow tissue sinuses where its subsequent main direction was toward the nearest major stream. This was especially noticeable in the appendages where complete circulation through them seemed to depend almost entirely on the aspirative force of the current that flowed perpendicularly across the opening of the appendage.

*Blood Flow in the Heart*

The heart closely resembled that of *B. trapezoideus* as described by Nutting (5), but the ventral, excurrent ostia were not observed. Possibly these ostia develop later in conjunction with wing development or they may simply be hidden from view by surrounding tissue in this preparation. The heart was strongly contractile from the caudal end to the cervical region. It aspirated blood from the loosely defined pericardial sinus through 12 pairs of lateral ostia. These were spaced regularly, approximately one pair for each body segment, from the last complete abdominal segment to the posterior part of the prothorax. Haemocytes entered the heart with the haemolymph. Most of them remained suspended but some adhered temporarily to the outer and inner walls and to the ostial valves. The blood was propelled anteriorly by peristaltic waves of contraction and passed through the aorta into the frontal sinus of the head. The aorta, or that portion of the dorsal vessel anterior to the first pair of ostia, was largely obscured from view by the muscles of the thorax and head, but there was no evidence that it assisted in the propulsion of blood by contraction. Cross sections of the embryo showed that it was suspended dorsal to the alimentary canal and opened into the head directly beneath the brain. Its opening was triangular in outline. The dorsal wall was appressed to the ventral surface of the protocerebrum and extended laterally to insert on dorsoventral muscles of the head. The ventral wall was drawn downwards and inserted on the wall of the pharynx. The pulse rate at this point coincided with the peristaltic contractions of the heart and was quite constant for each individual embryo. It ranged from 61 to 89 beats per minute. The average rate for 10 individuals at room temperature (approximately 23° C) was 72 beats per minute.

*Blood Flow in the Head*

Circulation was especially vigorous in the head and the streams there were better defined than in other regions. The streams, of course, had no real identity as vessels but are referred to here to indicate definite directions of flow and the separation of currents by major structures. In some cases, as in the sinuses immediately beneath the epidermis, between muscle bundles, or between muscles and organs they represented distinct channels wherein the

flow was comparatively rapid and unidirectional. On the other hand blood also percolated more slowly through narrow spaces among the tissues. It then had no clear boundaries but it maintained a principal direction and speed commensurate with the nature of the sinuses and its proximity to major streams.

Blood from the aorta poured forcibly against the concave anterior surface of the frontal sinus midway between and ventral to the bases of the antennae and literally streamed away in all directions. After following circuitous routes through the head, as described below in detail and as illustrated (Figs. 2 and 3), it rejoined dorsally behind the vertex and flowed out as a single stream directed ventrally through the cervix toward the perineural sinus of the thorax. There was no direct outflow of blood from the ventral part of the head as has been figured schematically by Wigglesworth after Brocher (1). Although this might be considered to be due to blockage of a ventral sinus because of the flexed position of the embryo's head, it would be true to some extent also for postembryonic stages of this hypognathous insect. Furthermore, an open sinus exists at this point beneath the oesophagus but carries a minor stream of blood inward to the head from the cervix rather than outward.

The brain received blood almost directly from the aorta. A major current flowed directly upward from the frontal sinus. It was separated by the brain into two streams, a clearly visible superficial one that moved over the face of the brain and another that disappeared from view as it flowed inward and dorsally over the posterior surface. They rejoined behind the vertex in the large, dorsal, cervical sinus.

Tissues in the clypeus, labrum, and mandibles were bathed by a superficial stream that flowed ventrally from the frontal sinus. In the mandibles, as in the other mouth-parts, the blood flowed distally in the anteromedial half and proximally in the posterolateral half. It then proceeded diagonally upwards through the genae, mingling with other streams leading to the cervical sinus.

The maxillae and labium were served by currents that flowed inward and ventrally from the frontal sinus around the pharynx. Occasional muscular contractions at the bases of the maxillary and labial palpi suggested the occurrence there of accessory pulsatile organs. However, no such organs were identified and the blood circulated slowly through the palpi when no contractions were occurring. Apparently circulation through these confined structures was effected almost entirely by propulsive and aspirative forces of the adjacent streams as described below for the antennae. Blood leaving the maxillae and labium flowed directly upward through the posterior portion of the genae to the cervical sinus.

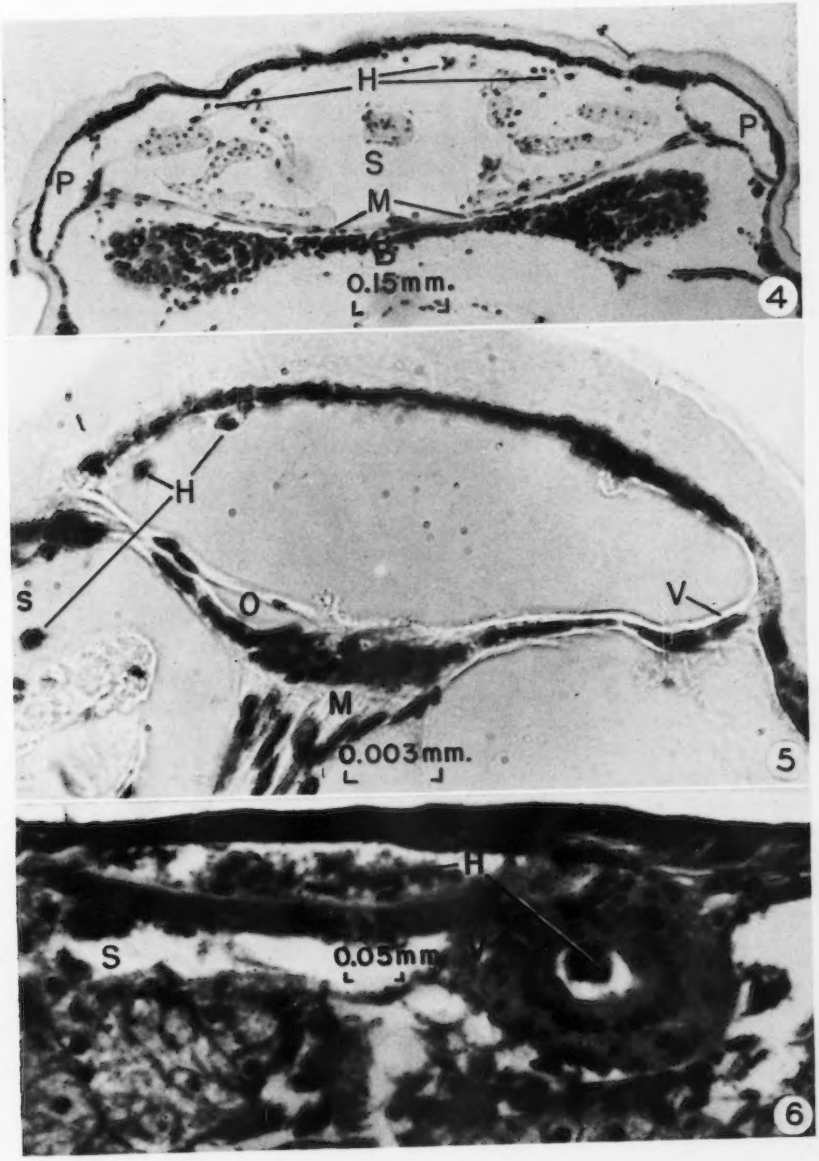
The compound eyes and the antennae received blood that streamed laterally from the frontal sinus through a relatively well-defined channel past the bases of the antennae. Part of this stream spread dorsally around the compound eyes to the cervical sinus and part of it entered the antennae. Although blood circulation in the antennae was the function of the frontal pulsatile organs, as described below, it persisted at a slow rate when these

organs were inactive and provided a clue to the mechanism of circulation in other appendages where pulsatile organs did not occur. Blood flowed distally in the anteromedial half of the antennae and proximally in the posterolateral half, the two currents being separated by a median membrane that extended into the penultimate segment. Apparently this circulation was effected, in the absence of action by the pulsatile organs, through aspiration of blood from the posterolateral sinus of the antennae by the current that streamed past the sinus outlet into the head, in addition to the small, direct flow of blood into the anteromedial sinus. This suggestion is based on three observations: (1) the bases of the antennae were situated at right angles to rapidly flowing streams of blood; (2) the blood pulse in the antennae corresponded to that in the head; and (3) the outlet from the antennae was much less obstructed by tissues and therefore more subject to aspiration than the inlet was to unassisted inflow. It seems unlikely that circulation would continue in the antennae without this combination of forces, especially when no obvious differential in pressure exists between the inlet and outlet. Possibly the same forces are responsible for circulation in other appendages which occur in similar situations and which undoubtedly offer resistance to blood flow because of their position outside of the main course of circulation and because of their constricted nature. Presumably this rather passive flow would be augmented in postembryonic stages by normal muscular movements.

Blood circulation in the antennae was caused normally by the so-called frontal or antennal pulsatile organs. These organs are known to occur in insects from several orders and have been cursorily described by a number of authors. They have been described in some detail for the honeybee *Apis mellifica* L. by Freudenstein (4), for the silkworm *Bombyx mori* L. by Schneider and Kaissling (8), for a number of Diptera by Clements (2), and for certain Orthoptera by Paulowa (6) and by Brocher (1). They appear to be fundamentally alike in the various insects but the lack of details concerning structure, the manner of communication with the haemocoel and with the antennae, or their effectiveness in antennal circulation makes broad comparisons difficult. In at least one case (Day (3)), roles other than circulation have been suggested for similar structures but seem unlikely.

In *B. giganteus* the frontal pulsatile organs comprised two hemispherical sacs, approximately 0.15 mm in diameter, in the lateral regions of the frontal sinus. They were attached to the cuticle of the frons near the bases of the antennae and were joined by a band of muscle (Figs. 2 and 4). The walls of each sac were composed of a single layer of cuboidal epidermal cells overlaid by a thin sheath of muscle fibers that spread from the muscle band and inserted

FIG. 4. Horizontal section through the head of the embryo showing pulsatile organs and connecting muscle bundle.
FIG. 5. Horizontal section through the pulsatile organ of the embryo.
FIG. 6. Horizontal section through the dorsal region of the pulsatile organ of an adult cockroach showing increased musculature and section of the blood vessel.
ABBREVIATIONS: B, brain; H, haemocytes; M, connecting muscle bundle; O, ostial valve; P, frontal pulsatile organ; S, frontal sinus; V, antennal blood vessel.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in the cuticle around the circumference of the sac (Fig. 5). A simple ostium in the wall of the sac opened dorsomedially into the frontal sinus, and a blood vessel whose walls were structurally similar to those of the sac emerged laterally. From observations on the active organ, the ostium appeared to be elliptical when the sac was contracted and circular when expanded. A short, thin-walled tube extended from the ostium into the lumen of the sac and was invested by a thin layer of muscle that inserted into the cuticle. The lateral blood vessel curved inward and dorsally from the sac and entered the antero-medial sinus of the antenna. It lost its identity in the second segment where it emptied into the sinus through which blood flowed to the tip of the antenna and was separated from the outflowing blood by the median membrane. There was no evidence of perforations in the median membrane as suggested by Paulowa (6) and by Brocher (1), or at least none of a size that permitted the passage of haemocytes, and the blood followed a complete circuit. The muscle band that joined the two sacs extended inwards and was firmly attached to the anterior surface of the brain just dorsal to the aorta opening. A connective strand also extended from this point on the muscle to the wall of the clypeus. In postembryonic stages of the insect the position of the pulsatile organs was marked externally by slight circular depressions in the cuticle beneath each ocellus. In the adult the sacs were approximately 0.5 mm in diameter and the blood vessel was about 3 mm long. Here the muscular sheath of the organ and its vessel was much thicker than in the embryo (Fig. 6) and showed circular as well as longitudinal fibers. The muscle sheath extended along the blood vessel only as far as the base of the antennae.

The action of the pulsatile organs was similar to that described for other insects by the previously mentioned authors. Contraction of the muscle band that joined the organs caused their expansion and the opening of the ostia. Blood from the frontal sinus, including haemocytes, entered at this time. Relaxation of the muscle band, presumably accompanied by contraction of the fibers that sheathed the sac and its ostia and blood vessel, resulted in contraction of the sacs, closure of the ostia, and expulsion of blood through the vessels into the antennae. The rate of action was relatively constant during the short periods of observation, but it varied with the individual. It seemed entirely unrelated to heart action. The average rate for 10 embryos was 25 beats per minute with a range of 14 to 40. Pulsation of the blood was identical at various points along the antennae and coincided with contractions of the pulsatile organs. This phenomenon demonstrated the role and the effectiveness of the mechanism.

*Blood Flow in the Thorax*

The blood was less confined in the thorax than in the head and flowed somewhat less vigorously. The main stream from the head was directed ventrally through a large, unobstructed sinus anterior to the thoracic muscles and entered the loosely defined perineural sinus. Along its course to the perineural sinus blood was also diverted elsewhere: a superficial stream flowed directly caudad from the head through the prothoracic shield and

into the anterior portion of the pericardial sinus; other currents swept around the crop and disappeared from view in the interior of the thorax; still other currents flowed caudad dorsal to the ventral diaphragm and percolated upwards over the thoracic muscles toward the pericardial sinus; a minor stream flowed back into the head beneath the oesophagus and rejoined the streams proceeding through the genae to the dorsal cervical sinus.

The blood in the perineural sinus flowed mainly caudad, but some was constantly diverted into the legs, which were appressed to the ventral surface of the body in a folded position. There was no evidence of accessory pulsatile organs, but the blood flowed distally in the legs through a much constricted anteroventral sinus and was separated by a median membrane from the blood flowing proximally in the posterodorsal portion. Apparently the median membrane was entire as far as the last tarsal segment and there was no visible cross flow of blood between the sinuses at any point along the circuit. Although circulation in the legs was complete and extended even into the tarsal claws, it was comparatively slow. This may account in part for the frequent loss of tarsi in ageing adults where the rate of circulation in general is declining and may cease altogether in such constricted regions. The fact that blood circulates in the legs in the absence of accessory pulsatile organs seems almost unaccountable considering the folded position of the legs in the embryo and the bulk of leg musculature in all stages. Brocher's (1) suggestion that it results from the pressure difference between the perineural sinus (high) and the perivisceral sinus (low), i.e. between the entrance into and the exit from the legs respectively, is probably true to some extent here and more so in insects with an unperforated ventral diaphragm. However, this difference in pressure must be small where the ventral diaphragm is rudimentary as is the case here and in many other insects. It seems possible, however, that circulation is effected in the legs as it is in the antennae when the frontal pulsatile organs are inactive. Thus blood is diverted into the ventral sinus of the legs from the perineural sinus and proceeds through them as a result of the aspiration of blood from the dorsal sinus by the current passing the exit. It is perhaps noteworthy that although accessory pulsatile organs ensure blood circulation in the antennae, which are primarily sensory, circulation in the legs is apparently unattended in this insect. On the other hand, in Hemiptera and perhaps in Lepidoptera where the legs have greater sensory roles, accessory pulsatile organs commonly occur in the legs.

*Blood Flow in the Abdomen*

The haemocoel in the abdomen was comparatively open and the rate and directness of blood flow decreased accordingly. The stream in the perineural sinus continued to flow caudad to the terminal segment and circled dorsally to the posterior end of the heart with little interruption. The principal diversion from it was into the short caudal cerci. Elsewhere the blood percolated slowly and unconfined among the viscera. Its steady onward movement was interrupted by the continual undulation of the viscera, but this seemed also to prevent stagnation of the blood among the organs and continually swept

the haemocytes from tissues where they tended to settle. The main direction of flow was diagonally upward from the perineural region to the pericardial region where the rate of flow increased again rapidly under the aspiratory influence of the heart.

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# PERMEABILITY OF THE EGG SHELL OF ACHETA DOMESTICUS (L.), AND THE FATE OF THE VITELLINE MEMBRANE[1]

J. E. McFarlane

## Abstract

The egg shell of *Acheta domesticus* (L.) is permeable to dyes in the interval between the fragmentation of the endochorion and the secretion of the lipoid layer of the serosal cuticle, i.e. at the beginning of the water absorption period. The vitelline membrane persists throughout the whole of embryonic development, lying between the aforementioned layers from the beginning of water absorption.

## Introduction

In a previous paper it was stated that the egg of *Acheta domesticus* (L.) was permeable to basic fuchsin and cresyl violet during the period of water absorption (3), which lasts from about the 36th to the 96th hour of incubation at 33° C. Subsequent work has now shown that the egg is, in fact, permeable to dyes only at the very beginning of the water absorption period.

## Observations

If newly laid eggs are immersed in a saturated solution of basic fuchsin the chorion stains very deeply with the dye, but examination of the yolk at any time *before* water absorption begins shows that no dye has penetrated the shell. Sections of these eggs (prepared by drying the egg in a desiccator and imbedding in paraffin) show an unstained vitelline membrane, but the chorion is almost uniformly very deep red. It is impossible to distinguish exochorion and endochorion except where these layers have separated, because of the extreme thinness of the latter (about 0.4 $\mu$ as against 2.5 $\mu$), and the fact that it takes up less stain than the exochorion. The staining of the endochorion is in fact so slight that it seems quite possible that the dye does not penetrate this layer, but is only adsorbed on the surface. At any rate, the barrier to dye penetration is in the region of the endochorion.

If eggs are immersed in saturated basic fuchsin *before* water absorption begins and are examined for evidence of yolk staining *after* water absorption has started, the yolk of most eggs is found to be stained. Sections of such eggs, prepared as above, show that in addition to the stained chorion there is a lightly staining thin layer at the surface of the otherwise *unstained* serosal cuticle; deep-staining spots about 0.8 $\mu$ in diameter, often showing fine processes which may connect adjacent spots, are distributed more or less uniformly throughout the layer (about 6 per 25 $\mu^2$). This layer is almost always found attached to the surface of the serosal cuticle, and is several

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tenths of a micron in thickness. At first this was thought to be the lipoid layer of the serosal cuticle (1). However, when stained eggs are treated with sodium hypochlorite, which is known to remove the chorion but to leave the lipoid layer of the serosal cuticle more or less intact (1), all of the stain is removed. Therefore, the stained layer on the serosal cuticle is not the lipoid layer; it most probably is the vitelline membrane. When stained eggs are immersed in sodium hypochlorite, this layer is often removed more or less intact, leaving an unstained serosal cuticle enclosing the egg; and as it shows no evidence of crystalloids, which might identify it as part of the chorion (1), it is unquestionably a separate layer. The vitelline membrane is found in the vacated shell.

In these stained eggs examined after water absorption has begun, the crystalloids of the endochorion can be seen where the exochorion and endochorion have separated. As before, the endochorion stains much less strongly than the exochorion; however, the crystalloids seem to stain more deeply than the unfragmented endochorion. So it is possible that the barrier to dye penetration does actually lie at the outer surface of the unfragmented endochorion. It may be recalled here that both the crystalloids and the unfragmented endochorion stain similarly with Mallory's (1). It is, however, possible that a chemical change in the endochorion enables it to take up more of the stain.

If eggs are immersed in saturated basic fuchsin at any time greater than 12 hours *after* water absorption has begun, no staining of the yolk or serosal cuticle results. So it appears that the shell is permeable to the dye only from the time the endochorion fragments to the time the lipoid layer of the serosal cuticle is formed. The serosal cuticle never stains, whether or not the yolk stains; therefore the shell must always be impermeable to the dye when it is surrounded by the serosal cuticle. Of interest is the fact that the semipermeability of the egg shell is a property of the lipoid layer of the serosal cuticle, not of the living serosa.

Failure of the endochorion to fragment in the normal way when eggs are immersed in a saturated tyrosine solution *before* water absorption begins (2) does not affect the permeability to basic fuchsin.

The finding of stained yolk in previous work (3) when eggs were immersed in dyes during the period of water absorption may now be explained. The eggs in those experiments were immersed after 36 hours of incubation, at a time when only a few of the eggs had begun to absorb water.

The experiments described in this work with saturated basic fuchsin were repeated with saturated cresyl violet, saturated ethyl eosin (alcohol), and 0.1% neutral red. Identical results were obtained. In all cases where the dye penetrated the shell, only the yolk was stained and not the embryo. The stain was taken into the gut with the yolk at engulfment and remained there until after hatching.

One further observation may be noted here. This is that most eggs immersed in basic fuchsin show a dark spotting of the exochorion which is due to the accumulation of the dye. These spots seem to correspond to the spots of

melanin which are found in the region of the endochorion when eggs are immersed in tyrosine (2). As it has been suggested that the melanized spots indicate the location of the ends of fine pore canals in the exochorion which are invisible in the light microscope (2), the spots of accumulated dye might therefore indicate the pore canals themselves.

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# PANAGRELLUS DUBIUS N. SP. (NEMATODA:TURBATRICINAE GOODEY, 1943), FROM FRASS OF BEETLE STERNOCHETUS LAPATHI (L.), WITH REMARKS ON REDESCRIPTIONS OF ANGUILLULA REDIVIVA (L., 1767)[1]

K. C. SANWAL

## Abstract

*Panagrellus dubius* n. sp., collected from frass of *Sternochetus lapathi* (L.) from Victoria, British Columbia, is described and figured. The new species differs from others of this genus in extent of bifurcation of the spicules and in having a lightly sclerotized supporting core in the velum of each spicule.

Redescriptions of "*Anguillula*" *rediviva* (Linnaeus, 1767) by Goodey (1922) and Ruehm (1956) are critically compared and the possibility of their dealing with two species is discussed. *P. dubius* is compared with *redivivus* as redescribed by Goodey and by Ruehm. Ruehm's synonymizing of *P. pycnus* Thorne, 1938, *P. leucocephalus* (Steiner, 1936) Goodey, 1945, and *P. silusiae* (de Man, 1913) Goodey, 1945 with "*Anguillula rediviva*" is not accepted.

Five male and four female worms were obtained from frass collected from the mines of *Sternochetus lapathi* (L.) received from Mr. J. W. E. Harris, Victoria, British Columbia. Close examination showed these to be of a new species of the genus *Panagrellus* Thorne, 1938.

## *Panagrellus dubius* n. sp.

*Male*

Length 0.76–1.01 mm (0.9 mm), $a$ = 26.2–30.3 (29.0), $b$ = 5.3–5.7 (5.4), $c$ = 8.4–10.1 (9.3), spicules 0.057–0.072 mm, gubernaculum 0.025–0.036 mm, ($n$ = 5).

Worms medium-sized and translucent. Body attenuated only very slightly toward anterior end. Posteriorly, body attenuated to a fine point beyond the cloacal aperture.

Cuticle smooth, without visible transverse striations; lateral fields marked with longitudinal lines, the exact number of which could not be determined. Head with six indistinct lips, not marked off from the rest of the body, and with six apical papillae (Fig. 1). Mouth opening wide. Stoma spacious; cheilorhabdions, which form the major part of the stoma, not appreciably sclerotized; prorhabdions prominent because of heavier sclerotization; meso-, meta-, and telo-rhabdions fused to form basal, funnel-shaped part of stoma. At junction of the prorhabdions with the walls of basal part of stoma, a toothlike structure is present, formed either dorsally or ventrally as an outgrowth of stomatal wall. The corresponding opposite side of stomatal wall appears to be normally sclerotized. (On a perfectly laterally mounted specimen, this toothlike structure is difficult to distinguish although the stoma gives the appearance of unequal sclerotization on the dorsal and ventral

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walls.) In addition to the dorsal or ventral toothlike structure, small irregular indentations appear on the wall of the funnel-shaped part of stoma.

Funnel-shaped part of stoma enveloped by esophageal tissue and opening into lumen of esophagus. Esophagus panagrolaimoid; corpus fusiform without distinct median bulb; isthmus about one-third as long as corpus. Terminal valvate bulb pear-shaped. Lumen of esophagus not straight. Radii of esophagus visible all along corpus. Cardia well developed. Lumen of intestine narrow, sinuous and uniform in diameter; walls of intestine filled with fat globules. Cloaca very small or absent, the terminal part of intestine and genital duct traceable separately up to anus, which is slightly elevated and is covered by a distinct cuticular flap. Rectal glands present.

Testis single, extending anteriorly beyond middle of body, where it is reflexed. Vas deferens very wide anteriorly, gradually narrowed for a short distance caudad and widened slightly just before joining a short, narrow, ejaculatory duct. Ends of intestine and genital duct not distinct.

Nerve ring surrounding isthmus; excretory pore on level with nerve ring or anterior to it.

Spicules two, large, and curved in the form of an open "C", lying parallel to each other. Each spicule with a smoothly curved, hook-shaped head that has notched area on the dorsal surface (probably for the attachment of muscles) (Fig. 4). Hook-shaped head varying slightly in shape. Shaft of uniform thickness with a narrow groove running along longitudinal axis and visible for about two-thirds of length of spicule from hooked end. Distal end of each spicule bifid for a distance equal to one-fifth to one-sixth of total length of spicule; two prongs distinctly separated and bluntly rounded at extremities. A velum extending from hooked end of spicule up to point of bifurcation and further reinforced by a definite, ridgelike, lightly sclerotized core, a little more than half as long as spicule. Gubernaculum long and apparently bifid at tip, lying closely apposed to dorsal surfaces of shafts of spicules.

Tail curved ventrally when relaxed by gentle heat (Fig. 5), narrowing gradually to a fine point. Tail region bearing seven pairs of caudal papillae as follows: two pairs preanal, subventral; two pairs postanal, subventral; two pairs postanal, subdorsal; one pair adanal.

*Female*

Length 0.98–1.2 mm (1.05 mm), $a$ = 25.1–30.7 (27.9), $b$ = 5.4–6.0 (5.6), $c$ = 9.2–9.5 (9.3), $V$ = 60–68% (65.2%), egg 0.041×0.028 mm ($n$ = 4).

General characters of cuticle, head, lips, cephalic papillae, stoma, esophagus, nerve ring, and excretory pore as in male. Tail (Fig. 3) tapering to a fine point

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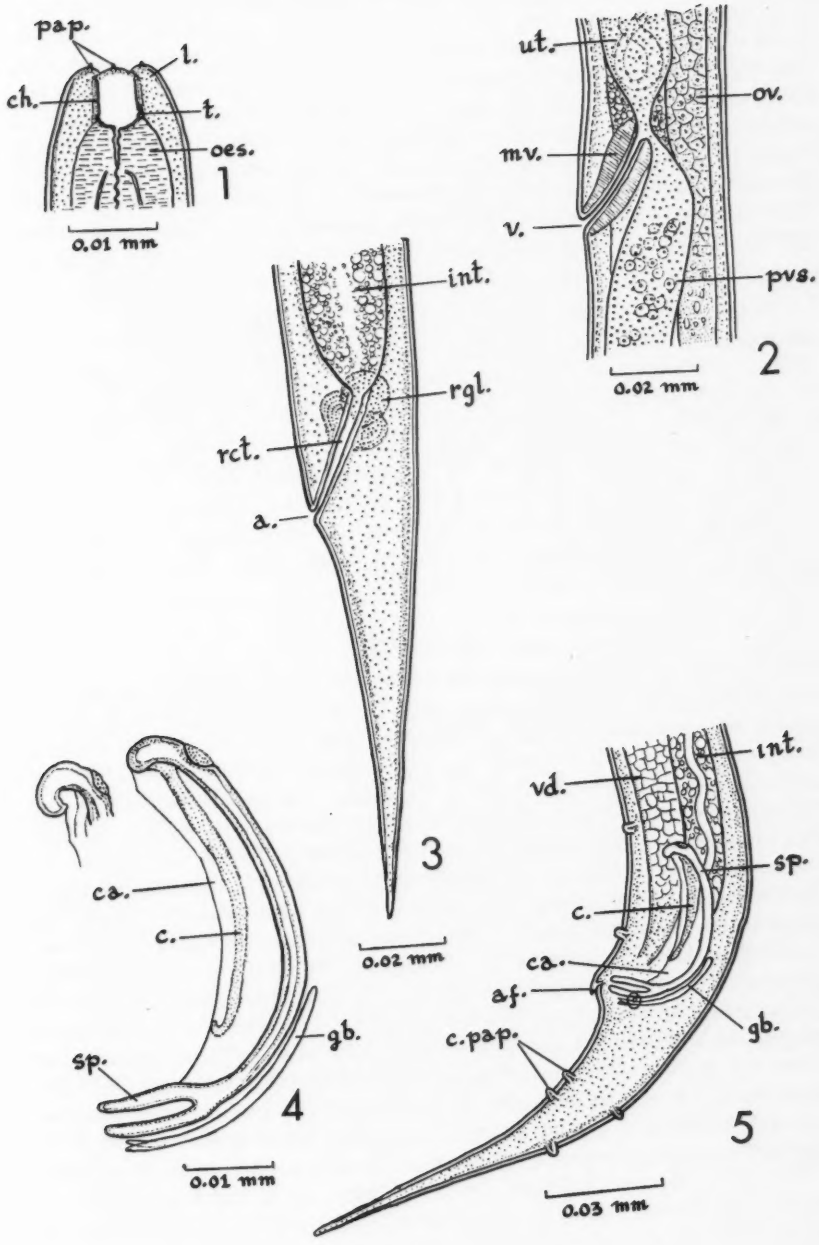
FIGS. 1–5. *Panagrellus dubius* n. sp. 1. Anterior region of male. 2. Vulvar region of female. 3. Tail region of female. 4. Spicule and gubernaculum in lateral view. 5. Tail region of male in lateral view.

ABBREVIATIONS: *a.*, anus; *af.*, cuticular flap; *c.*, sclerotized core; *ca.*, velum of spicule; *ch.*, cheilorhabdion; *c. pap.*, caudal papillae; *gb.*, gubernaculum; *int.*, intestine; *l.*, lip; *mv.*, muscular wall of vagina; *oes.*, esophagus; *ov.*, ovary; *pap.*, cephalic papillae; *pvs.*, postvulvar uterine sac; *rct.*, rectum; *rgl.*, rectal gland; *sp.*, spicule; *t.*, toothlike structure; *ut.*, uterus; *v.*, vulva; *vd.*, vas deferens.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but not curved ventrally as in male when relaxed by heat. Vulva postequatorial in position; in lateral view vaginal cleft running dorsocephalad (Fig. 2). Walls of vagina muscular and heavily cuticularized. Oblique vagina leading into wide uterus directed anteriorly. Uterus extending to about two to three bulb lengths posterior to esophageal bulb, where it is reflexed. Ovary long, slender, parallel to intestine, extending beyond vulva to about halfway between vulva and anus but not as far as rectum. A wide postvulvar uterine sac extending to halfway between vulva and anus. Uterus containing two to three eggs and numerous hatched larvae in specimens studied. Rectal glands present.

*Diagnosis*

*Panagrellus* with dimensions and general characters as described above. Distinguished from other species of the genus by the shape of the spicules, which are bifurcate at their distal ends for a distance equal to one-fifth to one-sixth of the total length of the spicule, and also by the presence of a lightly sclerotized supporting core in the velum of each spicule.

*Habitat.*—Frass from mines of *Sternochetus lapathi* (L.).

*Type locality.*—Victoria, British Columbia.

Holotype (male) and paratypes deposited in the Canadian National Collection of Nematodes, Nematology Section, Entomology Research Institute, Ottawa, Ontario. Collection No. 1600.

## Discussion

The generic name *Panagrellus* Thorne, 1938 (12) is used in this paper in favor of *Anguillula* O. F. Mueller, 1783 (7). This decision was made on the basis of the expressed opinions of Stiles and Hassall (11), Peters (8), Goodey (3–5), and Ruehm (9) on the validity of the generic name *Anguillula*.

The forms described above closely resemble *Panagrellus redivivus* (Linn., 1767) Goodey, 1945 (5). However, a complication arises on comparing them with *P. redivivus*. The species *redivivus* has been redescribed by Goodey (3) and by Ruehm (9). The descriptions are by no means identical and indicate some important morphological differences, as indicated below. Since it is not yet clear whether either Ruehm's specimens or Goodey's specimens represent *P. redivivus* (L., 1767), the forms described herein were compared with descriptions made by both authors. If future studies show that Ruehm's and Goodey's descriptions are of the same species, and that *P. redivivus* has a range of characters embracing both authors' descriptions, the species described herein will have to be synonymized with it. At present, however, Ruehm's specimens are evidently different from Goodey's and both of them different from those described in this paper. Accordingly these forms are treated as representing a new species, for which the name *Panagrellus dubius* is proposed.

The main difference between the descriptions of the species *redivivus* by Goodey and Ruehm are as follows: According to Goodey, the walls of the buccal cavity in optical section bear slight lateral thickenings and there are

no teeth or toothlike processes as in *A. aceti*, even when the worms are examined under oil immersion. Ruehm, in his description, speaks of "drei gleichgrosse Zahne ... eine Cuticularwarze ... jewils zwei Zanchen" in the buccal cavity. Since the morphology of the spicules and gubernaculum form the main basis for the differentiation of species in this genus, a close study of the observations and illustrations made by the above two authors indicated the following differences: Ruehm described and also illustrated a faintly notched, strongly cuticularized forward arching between the forked branches of the spicule; arching was absent in Goodey's specimens. Ruehm noted that the gubernaculum was split with a knob-shaped forward arching at the proximal end which does not agree with Goodey's description. Although Ruehm did not mention the size of ovary, it is to be noted that Goodey's specimens have a relatively small ovary, lying anterior to the uterus.

*Panagrellus dubius* differs from Goodey's *P. redivivus* in extent of bifurcation of the two spicules, possession of a slightly sclerotized supporting core in the velum of each spicule, length and size of ovary, relative size of isthmus and corpus, and presence of one large toothlike structure in the buccal capsule. The spicules in *P. dubius* are bifurcate for a distance equal to one-fifth to one-sixth of the total length of the spicule whereas in Goodey's specimens the bifurcation extends only up to 1/10th of the length of the spicule; the lightly sclerotized reinforcement of the velum present in *P. dubius* is lacking in Goodey's specimens; the ovary in the new species is long and reflexed beyond the vulva to a point near the anus in contrast to the short ovary lying in front of the uterus in Goodey's specimens; the isthmus in Goodey's specimens is relatively very short, the ratio between the length of the corpus to that of isthmus being 6:1 in contrast to the longer isthmus in *P. dubius*, in which the ratio is 3:1. Further, the distinct toothlike structure present on the walls of the stoma in the new species is absent in Goodey's specimens.

*P. dubius* differs from Ruehm's specimens in the presence of only one large toothlike structure in the stoma instead of five teeth (three large and two small) and a cuticular wart described by Ruehm. The shape of the spicules has been used to differentiate the species of this genus, and the following differences are evident between the spicules of *P. dubius* and those of Ruehm's *A. rediviva*. The forward-arched, sclerotized portion between the prongs of the spicules (a character also used in Ruehm's key to the species) is present in Ruehm's species but not in *P. dubius*. The sclerotized reinforcement of the velum present in *P. dubius* is absent in Ruehm's specimens. The knob-shaped forward arching at the proximal end of the gubernaculum described for *A. rediviva* of Ruehm is not present in *P. dubius*.

*P. dubius* resembles *P. zymosiphilus* (Brunold, 1950) Brunold, 1954 (1, 2), in the presence of a toothlike structure in the stoma, but the two species differ greatly in the shape and structure of the spicules and also in the size of the isthmus, which is very short in *P. zymosiphilus*.

*P. dubius* shows some resemblance to *P. silusiae* (de Man, 1913) Goodey, 1945 (6, 5). It differs, however, from *P. silusiae* in the absence of 'chitin'

stripes in the lip region, in the presence of the sclerotized core supporting the velum of each spicule, in the extent of bifurcation of the spicules, and in the spicules not being coalescent as in *P. silusiae*.

It should be mentioned here that the author does not agree with the synonymy of *P. pycnus* Thorne, 1938 (12), *P. leucocephalus* (Steiner, 1936) Goodey, 1945 (10, 5), and *P. silusiae* (de Man, 1913) Goodey, 1945 (6, 5) with *Anguillula rediviva*, as proposed by Ruehm (9).

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# A TECHNIQUE FOR REARING COCCINELLID BEETLES ON DRY FOODS, AND INFLUENCE OF VARIOUS POLLENS ON THE DEVELOPMENT OF COLEOMEGILLA MACULATA LENGI TIMB. (COLEOPTERA:COCCINELLIDAE)[1]

B. C. SMITH

## Abstract

A technique is described for rearing a coccinellid on a dry, particulate food. *Coleomegilla maculata lengi* Timb. (Coleoptera:Coccinellidae) was reared from first-instar larva to adult on the pollens of corn, gray birch, hemp, and hornbeam, and on the dried and powdered nymphs and adults of *Rhopalosiphum maidis* (Fitch.) (Homoptera:Aphididae).

## Introduction

*Coleomegilla maculata lengi* Timb. was the most abundant coccinellid predator on corn in the Belleville district when two of its known prey were present—the corn leaf aphid, *Rhopalosiphum maidis* (Fitch.) (Homoptera: Aphididae), and the European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera:Pyralidae). Britton (1) was the first to report that *C. maculata* eats pollen and fungus spores. Marlin (2) found that adults of this species would not eat corn pollen or pea aphids, *Macrosiphum pisi* (Harr.) (Homoptera: Aphididae), unless the pollen was offered on a piece of leaf from a corn plant in the tassel stage or the aphids were offered on alfalfa plants. The present author found corn pollen in the gut of field-collected larvae of *C. maculata* and other coccinellid species. This indicated that non-aphid foods may possibly influence the survival of predatory coccinellids. The present paper describes a technique for rearing adults and larvae of predatory coccinellids on dry foods, and also summarizes information on the development of larvae of *C. maculata* on various pollens.

## Materials and Methods

The larvae used in this study were the progeny of a number of female *C. maculata* that were confined in petri dishes in the laboratory at about 22° C and 65% R.H. These females were fed various non-aphid foods to be described elsewhere (Smith, in preparation). Larvae were fed the dead nymphal stages and adults of *R. maidis*. These stages were dried at 20° C and reduced to a fine powder. Larvae were also fed pollen of the following plants: corn, *Zea mays* L.; gray birch, *Betula populifolia* Marsh.; hemp, *Cannabis sativa* L.; hornbeam, *Carpinus caroliniana* Walt.; red pine, *Pinus resinosa* Ait.; and common ragweed, *Ambrosia artemisiifolia* L. The pollens were collected, dried at approximately 20° C, cleaned by sieving, and stored in darkness at 4° C until used.

[1]Manuscript received July 5, 1960.
Contribution from Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario.

A mated pair of *C. maculata* was reared in a cage (Fig. 1) consisting of a petri dish bottom containing a mat of filter paper, a slide with two cavities, and a perforated, disc-shaped cover grooved around its outer circumference to fit the bottom edge of the dish. The slide and cover were of plexiglass. The slide was 7.5 cm long and 2.5 cm wide and its cone-shaped cavities were each 16 mm in top diameter and 5 mm deep. Dry food was placed in one cavity and water in the other. A glass bead, 6 mm in diameter, was placed at the center of each cavity to reduce the chances of the insects becoming trapped in the food and water. The perforated cover prevented a buildup of humidity in the dish and thereby reduced the tendency of the dry food to form clumps.

Third- and fourth-instar larvae and pupae were reared in petri dishes as described for the adult except that the covers had smaller perforations. Larvae were confined individually because of frequent cannibalism. First- and second-instar larvae were reared in circular cells (Fig. 2) each 1.9 cm in diameter and 6 mm deep. The cells were formed by drilling 30 holes, six rows of 5 holes, in a piece of plexiglass 23.4 cm long and 18.4 cm wide. This perforated section was placed on a solid piece of plexiglass of the same dimensions that in turn rested on a piece of plate glass of the same dimensions, and the three sections were placed in a lidless box, 3 cm deep and enamelled white. Food was placed in one of two holes located in the floor of each cell and distilled water was placed in the other hole. The food hole was 5 mm in diameter and 6 mm deep (extended to the plate glass) and the water hole 3 mm in diameter and 4 mm deep. The top of the group of 30 cells was covered by six pieces of plate glass each 18.4 cm long and approximately 4 cm wide.

The eggs of the coccinellid were usually laid on the bottom of the petri dish cover; the incubation period was approximately 4 days. First-instar larvae were transferred to cells and given food and water within 6 hours of eclosion. During the first 2 days of life a small amount of water was deposited on the floor of the cells with a brush, but when the larvae were stronger and there was less danger of them being drowned the water holes were kept filled. Larvae being reared on the various foods were inspected at least once daily and records were kept of the duration of each stage. Larvae were transferred to clean cells or dishes after ecdysis or when appreciable amounts of frass had accumulated. The numbers of first-instar larvae started on each of the foods were as follows: 44 on powdered aphids; 214 on corn pollen; 60 on birch pollen; 94 on hemp pollen; 40 on hornbeam pollen; 50 on red pine pollen; and 30 on ragweed pollen.

## Results and Discussion

Adults that subsequently mated and produced fertile eggs developed from larvae reared on the powdered aphid and on all the pollens used except those of ragweed and red pine. First-instar larvae lived up to 2 days on ragweed pollen but did not increase in size or molt. There was no indication that this pollen was ingested. The pollen of red pine, though ingested, was not adequate for complete growth. On this food the durations of the first two

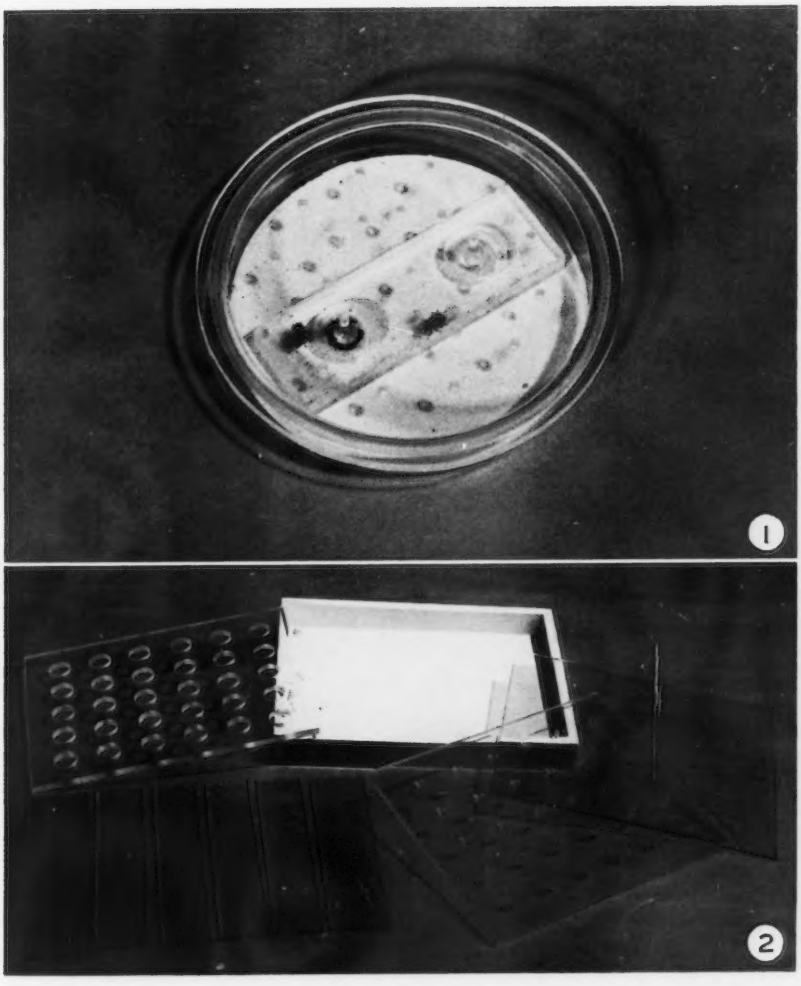

FIG. 1. Cage used to rear adults and third- and fourth-instar larvae.
FIG. 2. Parts forming the cells in which first- and second-instar larvae were reared.

larval instars were prolonged to 9 days for the first instar and to more than 8 days for the second. The normal durations of these stages are about 4 and 3 days respectively. There was no growth beyond the third instar on the pollen of red pine. Possibly pine pollen has inadequate protein content for normal growth: Nielsen, Grömmer, and Lunden (3) found that pollen of *Pinus montana* Mill. contained only 13% protein whereas other pollens studied, including corn, contained about 25% protein. No differences attributable to food were observed in the time required for the development of *C. maculata* from the first-instar larva to the adult stage on the powdered aphid and on the pollens of corn, birch, hemp, and hornbeam. The mean duration of this period for larvae reared on the powdered aphid and on these pollens was $23.86 \pm 1.58$ days. Survival to the adult stage of larvae reared on powdered aphids was 66% and did not differ significantly from survival on either birch pollen or hornbeam pollen. Survival of larvae reared on both corn pollen and on hemp pollen was significantly lower (chi-square, $P < .01$) than on powdered aphids.

If the food is nutritionally adequate the chief requirement for the growth of larvae of *C. maculata* on dry foods is that the particle size be sufficiently small to be ingested by the first-instar larva. It is not necessary that the food be offered with a closely associated plant, and it is not of primary importance that the food be one that the predator has previously experienced. *C. maculata* probably rarely, if ever, encounters powdered aphids or the pollens of birch, hornbeam, and hemp in the field. The first two pollens occur before the larvae of *C. maculata* are present and hemp pollen is so scarce locally that it is probably never encountered. Corn pollen is probably a factor in the survival of *C. maculata* as it is eaten by all stages and supports the growth of the larvae. The importance of corn pollen and other non-aphid foods in the survival of coccinellids will depend on the quality, quantity, and palatability of each in relation to the quality, quantity, and palatability of the animal foods present.

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# NEMATODE PARASITES OF VERTEBRATES OF EAST PAKISTAN

## VIII. ON ARTIONEMA LABIATO-PAPILLOSA (PERRONCITO, 1882) YEH, 1959 FROM THE BODY CAVITY OF HUMP BACK CATTLE[1]

S. P. GUPTA[2]


### Abstract

*Artionema labiato-papillosa* (Perroncito, 1882) from the body cavity of hump back cattle is redescribed. The species *Artionema labiato-papillosa* and *Artionema digitata* are considered synonymous.


### *Artionema labiato-papillosa* (Perroncito, 1882) Yeh, 1959

Figs. 1–15

*Synonym:* *Artionema digitata* (von Linstow, 1906) Yeh, 1959
*Material:* One male and 24 females
*Host:* Hump back cattle
*Location:* Body cavity

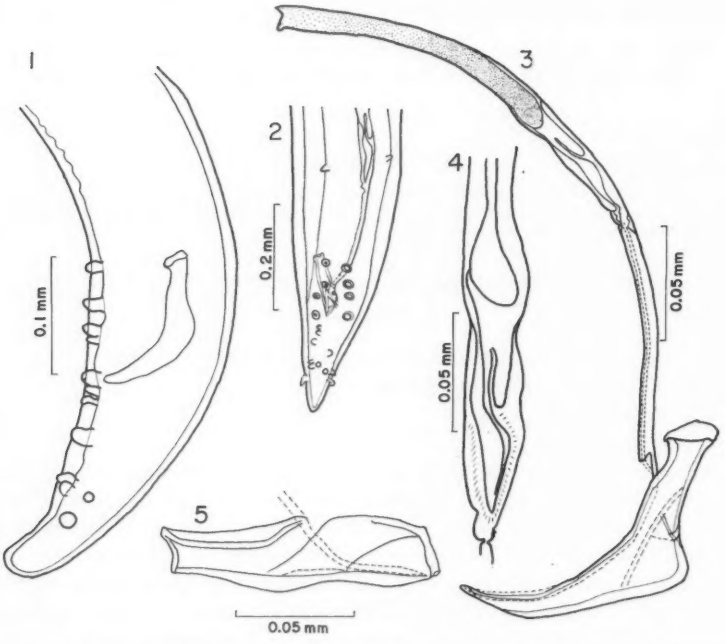
The genus *Setaria* has recently been divided into three genera by Yeh (6) viz. *Setaria*, in which the left spicule is stout and the head has four prominent spines: *Hyraconema* and *Artionema* in which the left spicule is slender and the head is without spines. The latter are differentiated by the presence of a long sclerotized membrane in *Artionema* and its absence in *Hyraconema*. Species of *Artionema* are confined to artiodactyles. There is, however, some doubt as to the validity of two of these species, *A. labiato-papillosa* and *A. digitata*. Purvis (3) and Baylis (1) considered them identical while Boulenger (2), Thwaite (5), Sarwar (4), and Yeh (6) regarded them as distinct.

An examination of 1 male and 24 female specimens from one animal has led the present writer to agree with Purvis and Baylis in regarding them as identical. Twenty of the female specimens had tails with a terminal knob, roughened with a papillated surface, while in the remaining four specimens the tail had a number of spines.

*Male*

The single male is 38 mm long by 0.4 mm wide but its measurements coincide with those given for both *A. labiato-papillosa* and *A. digitata*. The only differences are in the tail in which there is a single median preanal and three single postanal papillae (Fig. 2) and eight paired papillae: four preanal, one adanal and three postanal (Fig. 1). The anteriormost preanal papilla is near the anterior end of the shaft of the left spicule and the rest are spaced at regular intervals by the side of the right spicule. Of the preanal papillae,

[1]Manuscript received July 29, 1960.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada. The parasites upon which this paper is based were collected by Dr. Robert E. Kuntz (Cdr. MSC, USN: U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan), a member of the U.S. Naval Medical Mission to East Pakistan in 1958. He was assisted by James Reese, HM, IC, USN, and Charles Knight, HCM, USN, also of NAMRU-2. Dr. David H. Johnson, Curator, Division of Mammals, U.S. National Museum, identified the mammals.
[2]National Research Council, Postdoctorate Fellow.



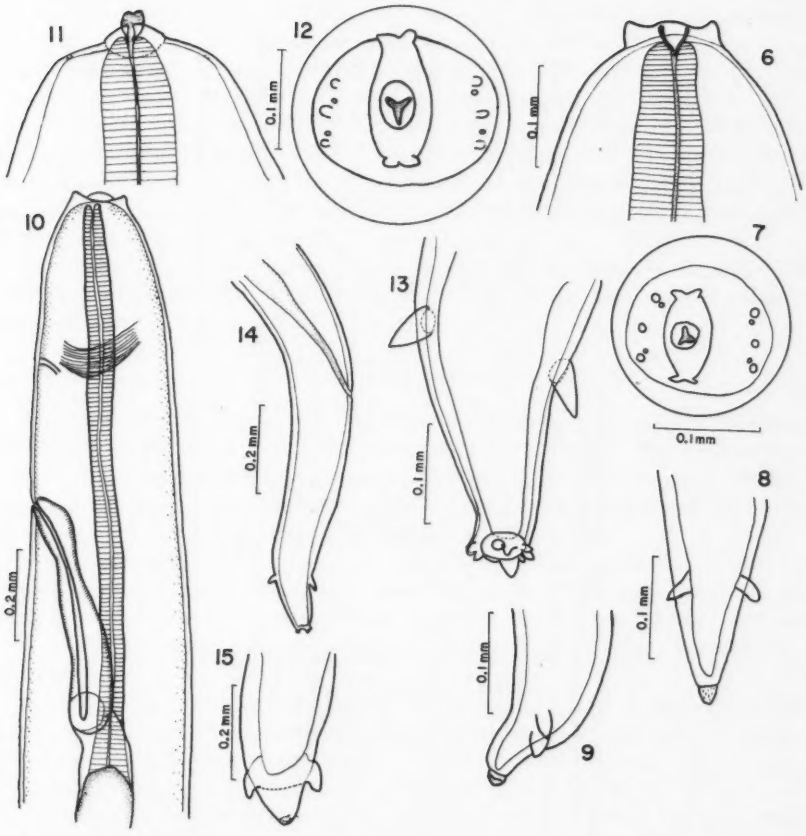

FIGS. 1–5. 1. Male tail, lateral view. 2. Male tail, ventral view. 3. Spicules. 4. Anterior portion of blade of left spicule. 5. Right spicule.

two small single papillae are just below the adanal and one is close to the posteriormost postanal papillae. Of the three paired postanal papillae, the median pair is the smallest, the other two pairs being of the same size as the preanal. The spicules are stout, unequal, and dissimilar. The left spicule (Figs. 3, 4) consists of a tubular shaft 0.36 mm long, twisted about half way down its length and with a tapering blade 0.33 mm in length protruding beyond the end of the shaft. The sclerotized membrane, 0.21 mm long, is usually overlooked. The right spicule (Fig. 5) is 0.12 mm long with the distal end bent over to form a groove. In both species there is one pair median, three precloacal, one adanal, and four postcloacal papillae. While the size of the right spicule in my specimen is within the size range of the others, the left spicule is half as long again (i.e., 0.36 mm as compared with 0.25–0.28 mm) and its blade is much larger (0.33 mm compared with 0.12–0.15 mm).

*Females*

The 24 females included 4 specimens in which the tail has a number of spines, while the remaining 20 have a terminal knob roughened and with a papillated surface. The two groups are described separately.

*Specimens without spines.*—50–68 mm long, 0.626–0.787 mm wide. In *en face* view (Fig. 7) mouth opening round, with inconspicuous buccal capsule, surrounded by a peribuccal crown; dorsal and ventral elevations, slightly




FIGS. 6–9. Females without spines. 6. Head, lateral view. 7. *En face* view. 8. Female tail, ventral view. 9. Female tail, lateral view.
FIGS. 10–15. Females with spines. 10. Anterior end, lateral view. 11. Head, ventral view. 12. *En face* view. 13. Female tail, ventral view. 14. Female tail, lateral view. 15. Terminal part of female tail, ventral view.

bifid (Fig. 6), 0.08–0.092 mm apart. Anterior part of oesophagus 0.643–0.939 mm long; posterior glandular part 5.13–6.9 mm long. Nerve ring and cervical papillae 0.26–0.295 mm and 0.425–0.469 mm respectively from anterior extremity. Excretory pore 0.28–0.3 mm from anterior end. Vulva in oesophageal region 0.452–0.539 mm from anterior end. *Tail* 0.382–0.522 mm long, diameter at anus 0.128–0.152 mm; ends in terminal spherical knob (Figs. 8, 9) roughened with a papillated surface. Conical caudal appendages 0.08–0.11 mm from posterior end.

*Specimens with spines.*—65–73 mm long by 0.696–0.785 mm wide. In *en face* view (Fig. 12) mouth opening oval with small buccal capsule, surrounded by a peribuccal crown; dorsal and ventral peribuccal elevation slightly

TABLE I

Comparison of forms placed in synonymy under *Artionema labiato-papillosa*
(all measurements in mm except where specified)

| | *A. labiato-papillosa* (female) (measurements from Yeh, 1959) | *Artionema digitata* (female) (measurements from Yeh, 1959) | Author's specimens | |
|---|---|---|---|---|
| | | | With spines (female) | Without spines (female) |
| Size | 60–94×0.6–0.9 | 65–75×0.5–0.7 | 65–73×0.695–0.785 | 50–68×0.626–0.787 |
| Dorsal and ventral elevations | — | 70–80 μ | 0.09–0.12 | 0.08–0.092 |
| Anterior oesophagus | 0.6–0.85 | 0.5–0.6 | 0.887–1.25 | 0.643–0.939 |
| Posterior oesophagus | 7.3–10.4 | 5.5–6.5 | 5.394–6.78 | 5.13–6.9 |
| Nerve ring | 0.25–0.29 | 0.2–0.3 | 0.33–0.38 | 0.26–0.295 |
| Cervical papillae | 0.46–0.63 | 0.4–0.5 | 0.37–0.425 | 0.425–0.469 |
| Vulva from anterior end | 0.45–0.8 | 0.5–0.6 | 0.52–0.68 | 0.452–0.539 |
| Tail | 0.44–0.60 | 0.4–0.5 | 0.4–0.46 | 0.382–0.522 |
| Lateral caudal appendages from posterior end | 0.09–0.13 | 60–70 μ | 0.09–0.1 | 0.08–0.11 |
| Knobs | Varying number of spines | Smooth or slightly roughened | Small spines | Slightly roughened |

bifid and 0.09–0.12 mm apart (Fig. 11). Anterior short muscular part of oesophagus 0.887–1.25 mm long, the glandular part 5.394–6.78 mm long. The nerve ring (Fig. 10) and cervical papillae 0.33–0.38 and 0.37–0.425 mm respectively from anterior extremity. Excretory pore 0.36–0.39 mm from anterior end. Vulval opening (Fig. 10) in oesophageal region 0.52–0.68 mm from anterior end. *Tail* 0.4–0.46 mm long with a diameter of 0.12 mm at anus; ends blindly in a spherical knob covered with an irregular circle of small spines (Figs. 13, 14, 15). The well-developed cone-shaped caudal appendages 0.09–0.1 mm from posterior end.

The table shows the important measurements of these two groups of females, compared with the measurements given by Yeh for *A. labiato-papillosa* and *A. digitata.*

## Discussion

The present forms closely resemble both *Artionema labiato-papillosa* (Perroncito, 1882) and *A. digitata* (von Linstow, 1906) but the male differs from them in the possession of an extra pair of preanal and three single median postanal papillae as well as three instead of four postcloacal papillae and in the possession of a left spicule which is half as much larger.

Some authors have considered the structure of the peribuccal crown in lateral view and the presence or absence of spines on the terminal knob as sufficient evidence for distinguishing between *Artionema labiato-papillosa* and *A. digitata*, while others consider them merely as variable characters in one species.

Boulenger (2) distinguished between them on the shape of the peribuccal crown in lateral view. He was of the opinion that in *A. labiato-papillosa* there is a prominent buccal crown much elongated dorsoventrally and deeply notched laterally, giving the appearance in side view of dorsal and ventral teeth separated by a wide depression, from the center of which arises a semicircular lip-like elevation. Each tooth in its turn is indented to form two cusps. For *A. digitata* he was of the opinion that the lateral notches are less deep and the lip-like chitinous projections are better developed and triangular. Thwaite (5) pointed out that in *A. labiato-papillosa*, lateral lips are crescentic and the female tail is tipped with an irregular circle of spines while in *A. digitata* the lateral lips are triangular and the female tail terminates in a smooth knob. Purvis (3) differs from Thwaite (5) in considering that the lateral lips of *A. digitata* are not triangular but vary from convexity to indentation. Spines at the tail vary from those described by Boulenger to a complete absence or replacement in certain individuals by an irregular surface or terminal knob. Sarwar (4) suggested that the shape and structure of certain parts of the peribuccal ring and chitinous lip-like projections are reliable as identification of the two species.

Yeh (6) in a comprehensive review of the genus *Setaria* distinguishes between *A. labiato-papillosa* and *A. digitata* on the following characters: (1) distance between dorsal and ventral elevations, (2) nature of mouth opening, (3)

distance of caudal appendages from hind end, and (4) absence or presence of spines on the terminal knob.

The author disagrees with Yeh (6) and considers that these forms are identical on the basis of the following characters:

(1) In the author's specimens the distance between the dorsal and ventral elevations is about 0.09–0.12 mm in specimens with spines and 0.08–0.092 mm in specimens without spines. In Yeh's specimens the distance between dorsal and ventral elevations averages about 0.075 mm in *A. digitata*; in *A. labiato-papillosa* they are anything from 50 to 100% farther apart. This means that the distance between dorsal and ventral elevations is more or less the same.

(2) In the author's specimens with spines, the mouth opening is oval with an inconspicuous buccal capsule, and in specimens without spines it is round. Yeh is of the opinion that in *A. digitata* the mouth opening is round and in the specimens of *A. labiato-papillosa* it is markedly elongated. In the author's collection no specimen was found with an elongated mouth opening. From this it is evident that the mouth opening is a variable character.

(3) In the author's specimens the distance of the caudal appendages from the posterior extremity is 0.09–0.1 mm in specimens with spines, and 0.08–0.1 mm in specimens without spines, while in Yeh's specimens it is 0.05–0.07 mm in *A. digitata* and 0.09–0.13 mm in *A. labiato-papillosa*. This means that the distance in *A. labiato-papillosa* is not twice that in *A. digitata*, as mentioned by Yeh, but more or less the same in both species.

(4) In the author's specimens the terminal knob is either provided with spines or shows some traces of wrinkling but is never perfectly smooth. Purvis and Baylis have also shown that specimens without spines are never perfectly smooth but are either an irregular shape or show some traces of wrinkling or shagreening.

From the above facts and information contained in the table it is evident that the differential characters suggested by Yeh (6) are too variable and thus unacceptable. Hence the author is forced to the conclusion that *A. digitata* and the form described here are synonymous with *A. labiato-papillosa*.

## Acknowledgments

The author wishes to express his gratitude for the personal interest taken by Professor T. W. M. Cameron and for his assistance and guidance throughout the preparation of the manuscripts in the series on East Pakistan material.

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# SOME FACTORS INFLUENCING THE FLIGHT ACTIVITY OF HYMENOPTEROUS PARASITES[1]

J. A. JUILLET

## Abstract

Analysis of the average captures of diurnal parasitic Hymenoptera in a pine plantation in relation to some weather elements showed that ichneumonids were most active at average temperatures (70–75° F), high relative humidity, and low wind velocity; braconids at high temperature, low relative humidity, and low wind velocity; and chalcids at high temperature and low relative humidity, but were unaffected by wind.

## Introduction

The flight activity of diurnal hymenopterous parasites varies from hour to hour depending in part on the weather conditions. Knowledge of the conditions governing this activity is required for a proper estimation of the value of a hymenopterous parasite in a given environment. This is a preliminary account on the influence of some weather elements on the flight activity of hymenopterous parasites as a whole.

## Material and Methods

Collections were made at the Waterloo County Reforestation Area, near Elmira, Ontario, during the summer of 1959. The area is a gently rolling 88-acre sandy tract planted mostly with red pine (*Pinus resinosa* Ait.) and Scots pine (*P. sylvestris* L.), but also some jack pine (*P. banksiana* Lamb.), white pine (*P. strobus* L.), white spruce (*Picea glauca* (Moench)), and European larch (*Larix decidua* Mill.). The pine trees are 10 to 15 years old and up to 15 ft high. A few old apple trees are also present. The area is divided into seven plots which are more or less delimited by roads or firebreaks. The red and Scots pine trees are infested by the European pine shoot moth (*Rhyacionia buoliana* (Schiff.)) and the European pine sawfly (*Neodiprion sertifer* (Geoff.)). Many other unidentified phytophagous insects such as aphids, leafhoppers, and loopers on pine, and tortricids and tent caterpillars on apple trees, were present in varying numbers.

One pure red pine plot was selected for the location of four rotary traps (Nicholls (1)). One trap was placed at the edge of the plot, along a road, and the other three inside the plot, in a partly open section, in a dense small-tree section, and in a dense high-tree section. Two levels of capture were selected, 4 and 6 feet. The traps were in operation from mid-May to mid-August and from 6 a.m. to 8 p.m. (E.S.T.), except when prevented by mechanical difficulties or bad weather. A total of approximately 540 million cubic feet of

[1]Manuscript received July 30, 1960.
Contribution from the Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario.

air was sampled. The traps were emptied every 2 hours, the insects killed, and the ichneumonid, braconid, and chalcid parasites were separated. The numbers of parasites captured are given per million cubic feet of air.

The temperature and relative humidity were recorded by a hygrothermograph installed in a clearing inside the plot, in the vicinity of the four traps; and the wind velocity from an anemograph placed on a crest in an adjacent plot.

## Results and Discussion

The average capture of hymenopterous parasites per million cubic feet of air during each of four periods of the summer was as follows:

| Periods | Ichneumonids | Braconids | Chalcids |
|---|---|---|---|
| Late May | 4.39 | 3.34 | 1.96 |
| Mid-June | 2.42 | 4.51 | 2.10 |
| Early July | 2.73 | 9.52 | 2.30 |
| Late July | 1.78 | 2.23 | 1.95 |

The average capture for the season was 2.72 ichneumonids, 5.03 braconids, and 2.11 chalcids. As the relations were similar in each period, the season is treated as one unit of time.

The three groups of parasites differ markedly in their diurnal cycle of activity (Fig. 1). Temperature and wind velocity are usually low in the morning and evening and high in the afternoon, and relative humidity is low in the afternoon and high in the morning and evening. Thus, it would appear from Fig. 1 that the daily cycle in the average capture of ichneumonids varies directly with relative humidity, and inversely with temperature and/or wind velocity; that the cycle of braconids does not follow the daily changes of any of the three factors; and that of chalcids varies directly with temperature and/or wind velocity, and inversely with relative humidity. The average captures of each group in relation to each of the three weather elements are illustrated in Fig. 2.

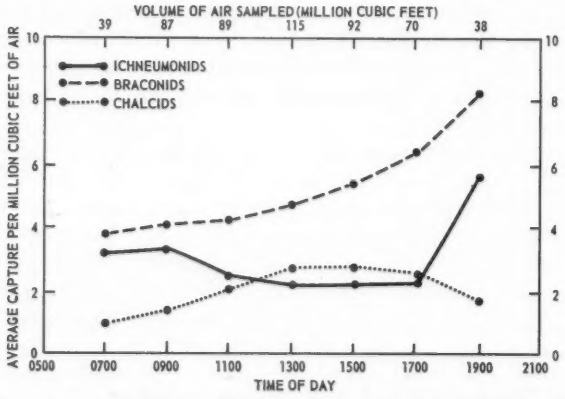


FIG. 1. Mean daily distribution of hymenopterous parasites captured in rotary traps during the summer of 1959 in a pine stand near Elmira, Ontario.

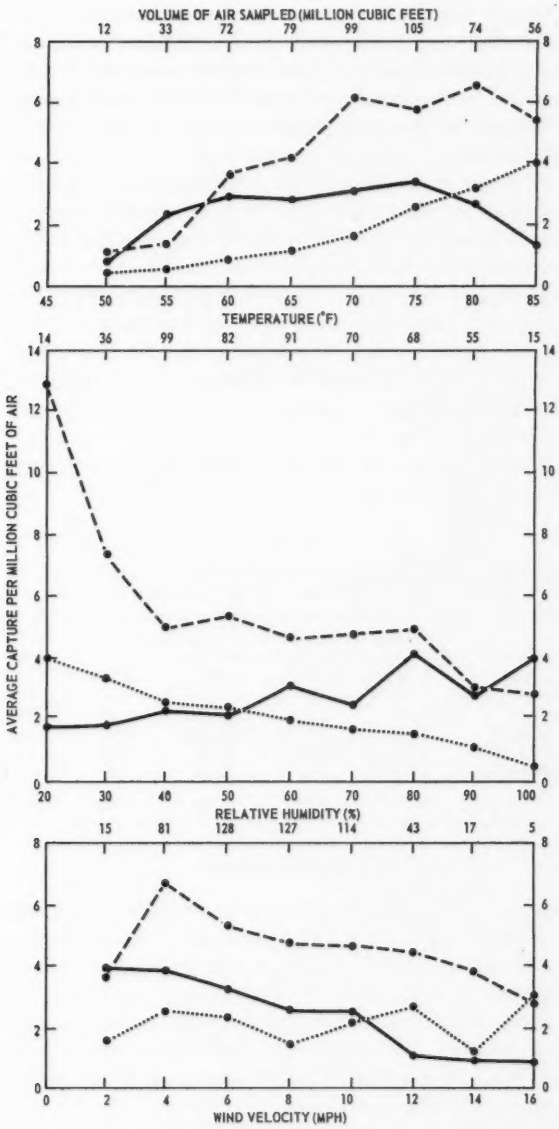
VOLUME OF AIR SAMPLED (MILLION CUBIC FEET)
12 33 72 79 99 105 74 56
TEMPERATURE (°F)
14 36 99 82 91 70 68 55 15
AVERAGE CAPTURE PER MILLION CUBIC FEET OF AIR
RELATIVE HUMIDITY (%)
15 81 128 127 114 43 17 5
WIND VELOCITY (MPH)

FIG. 2. Influence of some weather elements on the flight activity of hymenopterous parasites in a pine stand near Elmira, Ontario, during the summer of 1959.

The response of the ichneumonids to relative humidity and wind velocity agrees closely with the response expected from the trend in the daily cycle (Fig. 1), though there are great variations at high relative humidity. Contrary to expectations, however, the relation between temperature and ichneumonid activity is positive for temperature up to 75° F and negative above it. Factors other than temperature must depress activities of ichneumonids during the warm part of the day.

The braconids respond directly to temperature and inversely to relative humidity and wind velocity. In the morning, temperature and relative humidity are unfavorable but wind velocity is favorable and the capture is minimum; in the afternoon, the first two are favorable but not the third; and in early evening, all three are favorable and the capture is at its peak (Fig. 1).

The response of chalcids to temperature and relative humidity agrees well with the response expected from the daily cycle (Fig. 1). On the other hand, the wind velocity appears to have no influence on the flight activity of this group as there were as many chalcids caught at high wind velocities as there were at low velocities (Fig. 2).

All three groups are most abundant at low levels at the edge of the stand and least abundant at low levels in the dense high-tree section (Table I).

TABLE I

Average capture of hymenopterous parasites per million cubic feet of air at two levels of each of the four trap locations

| Locations | Ichneumonids | | Braconids | | Chalcids | |
|---|---|---|---|---|---|---|
| | 4 ft | 6 ft | 4 ft | 6 ft | 4 ft | 6 ft |
| Edge of stand | 4.75 | 2.83 | 8.22 | 7.28 | 4.03 | 2.39 |
| Partly open section | 2.48 | 1.80 | 5.04 | 7.16 | 2.39 | 2.23 |
| Dense small-tree section | 3.53 | 1.09 | 4.22 | 3.98 | 1.40 | 1.64 |
| Dense high-tree section | 0.79 | 4.49 | 1.31 | 3.03 | 0.74 | 2.06 |
| Average | 2.89 | 2.55 | 4.70 | 5.36 | 2.14 | 2.08 |

## Conclusions

Though each group of parasitic Hymenoptera studied here showed a definite trend in their daily cycle, it is not a necessary conclusion that one species responds in the same general manner as the group to which it belongs. For example, the braconid *Orgilus obscurator* (Nees), a parasite of the European pine shoot moth, was most active in the morning and least active in late afternoon and evening. This is a reverse condition to the average response of the group. Thus, the conclusions drawn here must of necessity be generalized and limited to each group as a whole.

The density of the vegetation in the study area varies from bare ground on the roads; from thin to heavy grass growth on or beside firebreaks; and from open to dense tree growth in large plots over the remaining part of the area. Thus the conditions favored by any one group can be met in one or many of the above situations.

The following general indications may be drawn from this study.

(1) Ichneumonids, which tend to be relatively large insects capable of directed flight, tend to be most active where the vegetation is fairly dense. In such a situation, one expects a microclimate with a low temperature or more uniform temperature, high relative humidity, and low wind velocity.

(2) Braconids, which tend to be moderate-size insects capable of directed flight, tend to be most active where the vegetation is partly open with a high temperature, low relative humidity, and low wind velocity.

(3) Chalcids, which tend to be small insects carried readily by air currents, tend to be most active in open or partly open vegetational growth where the temperature is at its maximum, the relative humidity at its minimum, and the wind velocity is variable, as this last factor appeared to have no influence on the flight of this group.

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# EFFECTS OF INITIAL DENSITIES AND PERIODS OF INFESTATION ON THE GROWTH-FORMS OF A HOST AND PARASITE POPULATION[1]

T. BURNETT

## Abstract

Initial densities and periods of infestation of adults of *Trialeurodes vaporariorum* and its chalcid parasite *Encarsia formosa* influence subsequent fluctuations in numbers of adults of both host and parasite.

Previous experiments with the propagation of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westw.), and its chalcid parasite *Encarsia formosa* Gahan showed that the numbers of adults of the host and parasite species fluctuated with peaks of slightly increasing magnitude (1). But there was considerable variation among the growth-forms of eight populations, chiefly with respect to the average densities of host and parasite, to the magnitudes of the fluctuations of host and parasite, and to the sequence of large and small fluctuations. The fluctuations obtained in each experiment, however, appeared to be related not to chance events but to two aspects of the manner in which the experiment was started. First, the average density of the host population in each experiment appeared to be dependent on the density of the host at the beginning of the experiment: when initial fluctuations were low subsequent fluctuations were relatively low and when initial fluctuations were high subsequent fluctuations were relatively high. And, as the numbers of adult parasites tended to vary, with a lag, as the numbers of adult hosts, the average density of each parasite population was related to its initial density. Second, the growth-forms of *T. vaporariorum* and *E. formosa* appeared to vary markedly with differences in the age-structure of host and parasite populations: populations with initially identical numbers of adults of host and parasite but with different larval (both parasitized and unparasitized) densities showed pronounced differences in the subsequent fluctuations of adults of host and parasite. If these two aspects of the interaction of *T. vaporariorum* and *E. formosa* are substantiated it is likely that many different growth-forms of this host and parasite population occur and that each results from the conditions prevailing at the outset of the interaction. If this proves to be the case, it is necessary to control the type of growth-form used for a detailed analysis of the interaction of *T. vaporariorum* and *E. formosa* so that this interaction may be compared with those obtained by other workers for these two species and for other host–parasite systems.

In many cases the influence of initial densities and age-structures on the interaction of two populations may be determined by infesting the experimental areas simultaneously with appropriate numbers of the various stages

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of each species. However, all the immature stages (with the exception of part of the first larval stage) of *T. vaporariorum*, and consequently all the immature stages of its internal parasite, are fastened on the underside of the leaf of the host plant. In this case, to vary the initial densities of host and parasite and to modify the synchronization between the relative abundance of host larvae and parasite adults, it is necessary to infest the host plants with various numbers of adult hosts and parasites for various periods of time.

This paper deals with the effects of initial densities and periods of infestation of adult hosts and parasites on the subsequent fluctuations in the numbers of adults of *T. vaporariorum* and *E. formosa* propagated on tomato plants in the greenhouse.

## Materials and Methods

All stages of the greenhouse whitefly, *T. vaporariorum* (family Aleurodidae, order Homoptera) occur on the underside of the leaves of the host plant. Except for the first larval instar all the immature instars are sedentary. Male and female adults appear in approximately equal numbers. The parasite, *E. formosa* (family Eulophidae, order Hymenoptera), lays its eggs through the dorsal surface of the quiescent larval instars of the host. The parasite, which reproduces parthenogenetically, has been reared continuously at this laboratory since 1929.

Eight populations of *T. vaporariorum* and *E. formosa* were each reared on 80 plants of tomato, *Lycopersicon esculentum* Mill., in greenhouse rooms 14 ft by 17 ft in area. The 80 host plants were divided into four lots of 20 each; each lot was introduced into the experimental area one-half month after the previous lot. When placed in the greenhouse each plant had about six leaves. Plants were allowed to grow 1 month and were held without further new growth for a second month. They were then placed under the greenhouse benches for 2 weeks to permit the emergence of any parasites they might contain. The host plants were arranged on the greenhouse benches in groups of four, one plant belonging to each of the four age lots. A young plant was added and an old plant removed from each group every half-month. The position of each plant in each of the 20 groups was chosen at random. The 20 groups of host plants were placed on the north, west, south, and center benches of each greenhouse section (I, Fig. 1). For purposes of sampling they were divided into five blocks of four groups each—the north, west, south, east, and center blocks. The east block contained the end groups on the north, center, and south benches. The number of adult hosts and parasites were counted, twice a week, on one of the groups of four plants in each of the five blocks. The group used for sampling was chosen at random from the four groups in each block and was used throughout the experiment.

The temperature at which the populations were reared was regulated by thermostatic control, an electric fan which produced a gentle circulation of air, shading of the greenhouse room when required, and automatic ventilation with temperature rise. At any given time the temperature in each unit was maintained, horizontally and vertically, within 1 degree F. Throughout the

TABLE I

Schedules of infestation of adult hosts and parasites

| Population | Host | | | Parasite | | |
|---|---|---|---|---|---|---|
| | Total number introduced | Number of introductions | Infestation period (days) | Total number introduced | Number of introductions | Infestation period (days) |
| I | 1500 | 5 | 1–16 | 1000 | 5 | 16–29 |
| J | 3000 | 5 | 1–16 | 2000 | 5 | 16–29 |
| K | 3000 | 5 | 1–16 | 2000 | 5 | 16–29 |
| L | 6000 | 5 | 1–16 | 4000 | 5 | 16–29 |
| M | 1250 | 5 | 1–15 | 900 | 5 | 15–30 |
| N | 1250 | 10 | 1–33 | 900 | 10 | 15–47 |
| O | 1250 | 10 | 1–33 | 900 | 5 | 15–30 |
| P | 1250 | 5 | 1–15 | 900 | 10 | 15–47 |

experiments temperatures fluctuated between 72 and 76° F, with an average value of 73–74° F.

There were no immature forms present on the host plants before they were placed in the experimental units. The infestation schedules for the eight populations are given in Table I. The first four populations, called I, J, K, and L, were started in early September and propagated simultaneously in adjoining sections of greenhouse. These populations were begun at three different densities of host and parasite adults—two populations being replicates—but with equal periods of infestation. Thus, population I was started by introducing 300 adults of *T. vaporariorum* into the experimental section on five occasions between the 1st and 16th day of the experiment and 200 adults of *E. formosa* were introduced on five occasions between the 16th and 29th day. The remaining four populations, called M, N, O, and P, were started a year later and propagated simultaneously. All four populations were started with equal numbers of adult hosts and equal numbers of adult parasites but the period of infestation and the number of infestations in each period were varied. In populations M and P 250 adults of *T. vaporariorum* were introduced on five occasions between the 1st and 15th day whereas in populations N and O 125 adults were introduced on 10 occasions between the 1st and 33rd day. In populations M and O 200 adults of *E. formosa* were introduced on four occasions and 100 adults on one occasion whereas in populations N and P 100 adults were introduced on eight occasions and 50 adults twice between the 15th and 47th day.

Species other than *T. vaporariorum* and *E. formosa* were picked off the host plants when observed.

## Results and Discussion

*Initial Densities*

The fluctuations in the numbers of adult hosts and parasites observed in populations I, J, K, and L are shown in Figs. 1, 2, 3, and 4 respectively. The first census of each population was made either 4 or 5 days after the last introduction of adult parasites into the experimental units. Figures 1–4 show

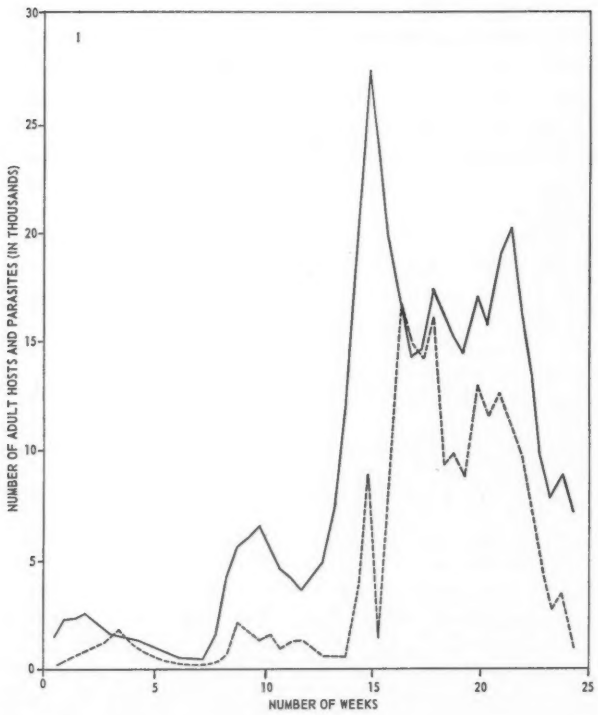


FIG. 1. Numbers of adult hosts and parasites counted in population I (initial densities: 1500 hosts, 1000 parasites). Solid line, hosts. Dotted line, parasites.

that in all four populations the numbers of adult hosts and parasites fluctuated with both the successive maximal and minimal values increasing in magnitude and that the fluctuations in each population occurred at approximately the same time. But whereas the general trend of the variation in numbers was similar in the four populations the densities of host and parasite varied among the experiments. At each of the 49 census periods, the number of adult hosts recorded in population I was less than the numbers recorded for either population J or K. And the number of hosts recorded in population L was greater than those recorded in populations J or K, except for six consecutive counts in population J near the 15th week and three consecutive counts in population K near the 15th week. The graphs of the numbers of host adults in populations J and K (Figs. 2 and 3) are almost identical, except for slight differences at the first and second peaks and greater differences from the 18th to 20th weeks. The numbers of adult parasites followed the usual pattern of increasing, with a lag, as the host numbers increased, decreasing to a very low minimum, and then fluctuating at first slightly and later to a much greater extent. The magnitude of the fluctuations were related to the

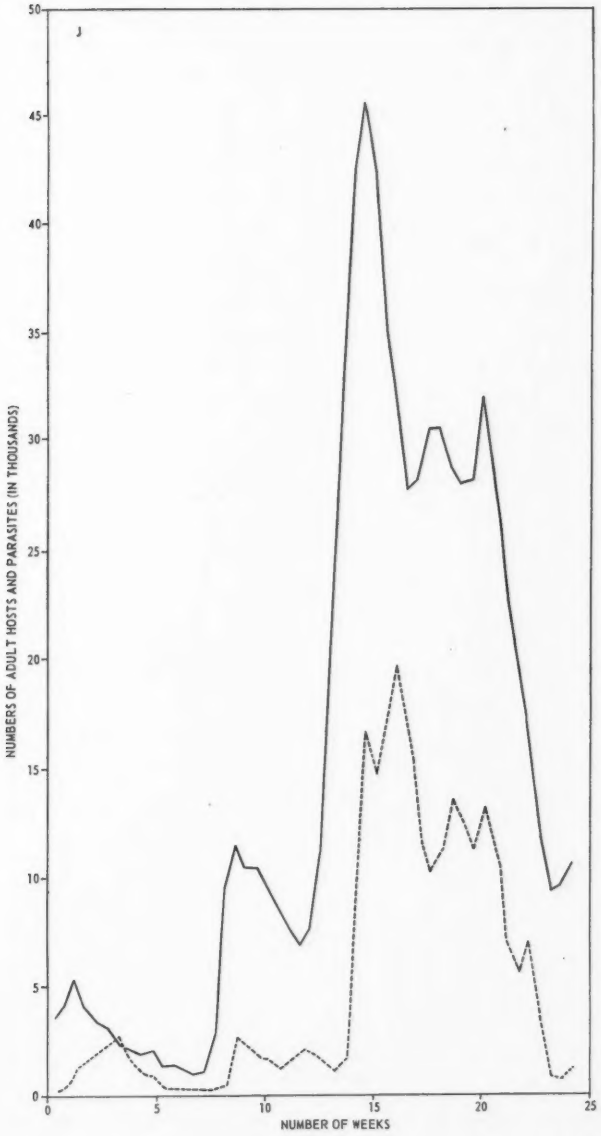


FIG. 2. Numbers of adult hosts and parasites counted in population J (initial densities: 3000 hosts, 2000 parasites). Solid line, hosts. Dotted line, parasites.

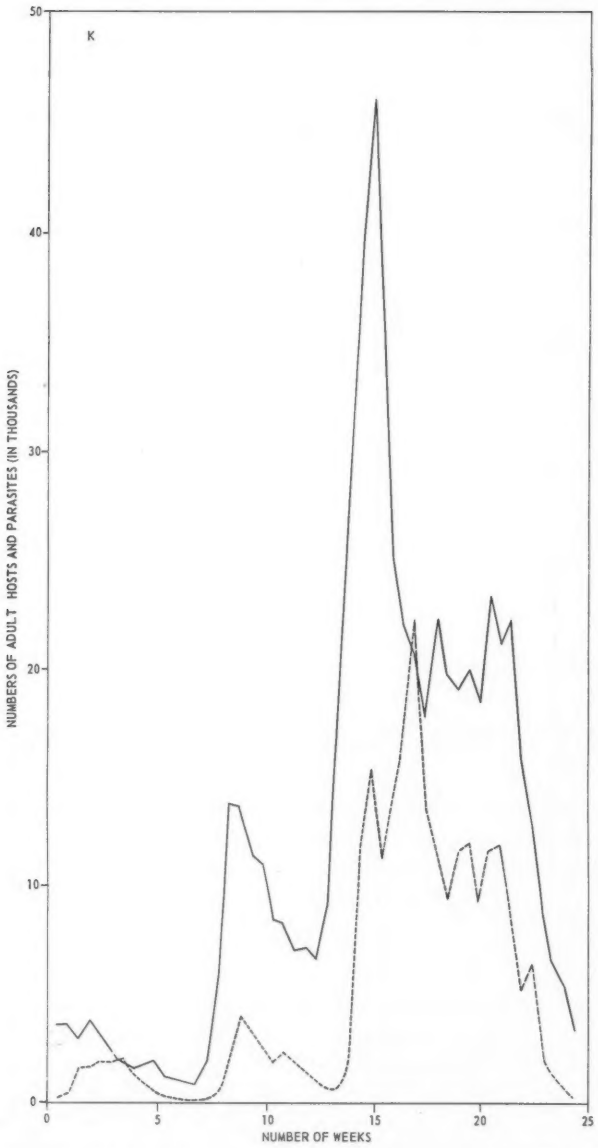


FIG. 3. Numbers of adult hosts and parasites counted in population K (initial densities: 3000 hosts, 2000 parasites). Solid line, hosts. Dotted line, parasites.

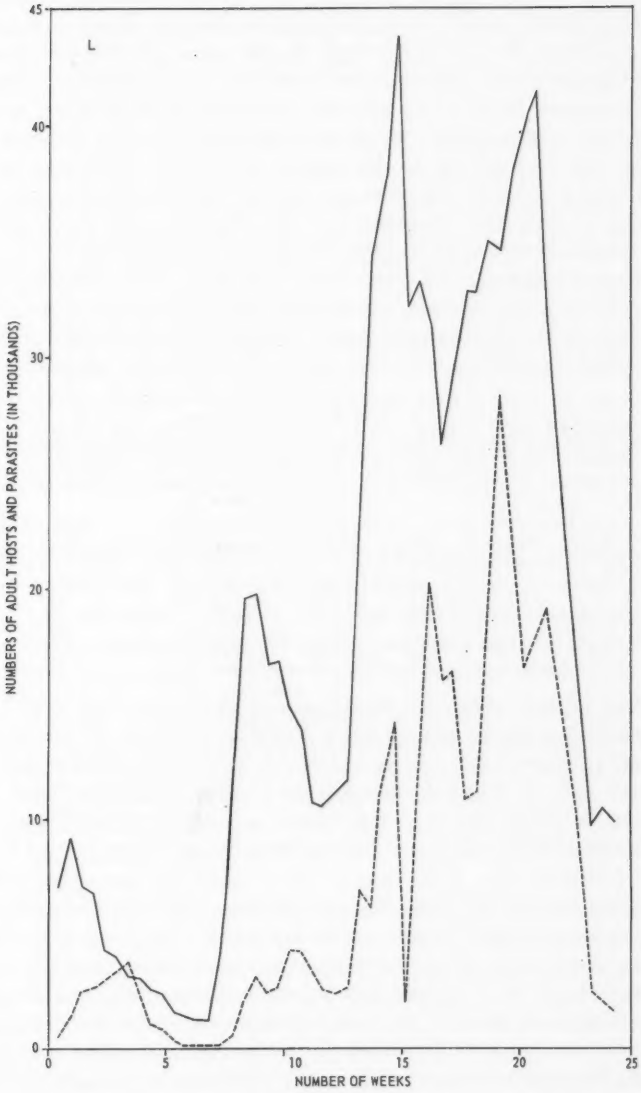


FIG. 4. Numbers of adult hosts and parasites counted in population L (initial densities: 6000 hosts, 4000 parasites). Solid line, hosts. Dotted line, parasites.

magnitude of the fluctuations in host numbers but in all four populations the peaks of parasite numbers were considerably less than those of corresponding host numbers. An unusual feature of the growth-forms of the parasite was the sharp decrease in populations I and L that occurred at the 15th week.

As the numbers of host and parasite fluctuated with peaks of increasing magnitude the nature of the fluctuations obtained may be summarized by listing the maximal values in the variations of host and parasite adults. Maximal values are not, of course, an accurate measure of variation among populations because they measure only small portions of the growth-form and because the host has such a high rate of increase small differences in minimal numbers are greatly magnified at the following peak. But they give an indication of the general trends in the growth of host and parasite populations. The averages of three consecutive counts of adults made at each of the three successive maxima in the numbers of host and parasite are given in Table II.

TABLE II

Average number of adults (in hundreds) in populations I–L determined from three consecutive counts made at successive peaks of host and parasite fluctuations

| | I | | J | | K | | L | |
|---|---|---|---|---|---|---|---|---|
| Peak | Host | Parasite | Host | Parasite | Host | Parasite | Host | Parasite |
| First | 23 | 15 | 44 | 22 | 33 | 17 | 78 | 32 |
| Second | 60 | 15 | 104 | 17 | 112 | 30 | 168 | 37 |
| Third | 237 | 140 | 434 | 177 | 398 | 175 | 380 | 227 |

Table II shows that the average numbers of adult hosts which occurred at three successive peaks in populations J and K were intermediate between the corresponding numbers of populations I and L, except at the third peak of populations J, K, and L. And the average numbers of adult parasites of the three peaks of population J and K were somewhat greater than the corresponding values for population I and less than those for population L.

In three populations, J, K, and L, the number of adult hosts recorded reached a common level, approximately 45,000, about the 15th week of the census in spite of distinct differences among the growth-forms of the first two and latter populations up to this time. In all three populations the numbers of immature forms of *T. vaporariorum* were so large at the end of 15 weeks that many leaves of the host plants were destroyed. With the destruction of tomato leaves many host larvae, both parasitized and unparasitized, were destroyed. The upper densities of host and parasite in populations J, K, and L, therefore, resulted not from the interaction of the two species but from the limitation in the number of insects which the host plant could support.

The host and parasite adults were introduced into the experimental units in the proportion of one (population I) to two (populations J and K) to four (population L). Figures 1–4 show that the more hosts initially present the higher the first, second, and third peaks of subsequent fluctuations (with

exception of the third peak in numbers of hosts in population L) and that the fluctuations of the parasite varied in magnitude in relation to those of corresponding host fluctuations. The second peak of parasite numbers was sometimes equal to or less than that of the first peak, but this follows the pattern of previous experiments (1); it may be that a complete cycle of parasite numbers consists of two fluctuations, a smaller followed by a larger. The increasing densities of host and parasite within populations are indicated in Table II, which shows that in populations I, J, and K the numbers of adult hosts increased 10- to 12-fold and the numbers of adult parasites 8- to 10-fold, from the first to the third peak. The variation of host and parasite densities among populations is also listed in Table II. The average values of host numbers increased from population I to populations J and K to population L (except for the third peak), and at the second peak (9th to 10th week) were in the proportion of somewhat less than one to two to three. The average values of parasite numbers also increased from population I to populations J and K to population L, but the values for the largest population at each of the three peaks were only a little more or less than twice the corresponding values for the smallest population.

*Infestation Periods*

As shown in Table I, an equal number of adult hosts and an equal number of adult parasites were used to start populations M, N, O, and P but the periods during which the infestations of the four populations were made were different. The fluctuations in the numbers of hosts and parasites recorded in populations M and N are shown in Fig. 5 and those in populations O and P are shown in Fig. 6. For brevity, the periods of infestation of both the host and parasite species are called "short" periods if the adults were introduced into the experimental units over a 15–16 day period and "long" if introduced over a 32–33 day period. Because of the difference between the short and long periods of parasite infestation the census of populations N and P was commenced about two and one-half weeks after the first census of populations M and O.

Figures 5 and 6 show that in populations M, N, O, and P the numbers of adult hosts and parasites fluctuated with peaks of increasing magnitude. Two of the peaks of host density (population P, 16th week; population M, 20th week) were of sufficient magnitude that the host plants were slightly damaged, but these occurred relatively late in the interaction and even then did not exert a profound influence on the subsequent results. All four populations, in addition to exhibiting fluctuations of increasing magnitude, had much the same interval between peaks of host numbers—if peaks are judged subjectively—and there were only slight differences in the intervals between the peaks in parasite numbers. But in spite of these similarities in the fluctuations in the numbers of adult hosts and parasites in populations M–P, the magnitude of the fluctuations were vastly different, ranging, for example, from a maximum host density in population N of approximately 3800 to a

maximum host density in population P of about 33,000. There were a number of aspects in which the growth-forms of host and parasite populations varied with periods of host and parasite infestation. First, with long host introductions and with both long and short parasite introductions (populations N and O) the numbers of hosts and parasites remained initially stable for longer periods of time than with short host introductions (populations M and P). Second, with long host introductions and with both long and short parasite introductions (populations N and O) the average densities of host and parasite and the magnitudes of peak numbers of host and parasite were lower than with short host introductions. Third, the highest and lowest average densities and fluctuations occurred with a long period of parasite introduction (populations N and P) while a short period of parasite infestation (populations M and O) led to intermediate levels of host and parasite numbers. Fourth, with long host and long parasite (population N) and with short host and short parasite (population M) introductions two consecutive fluctuations of the host were of equal magnitude, whereas with unequal infestation periods

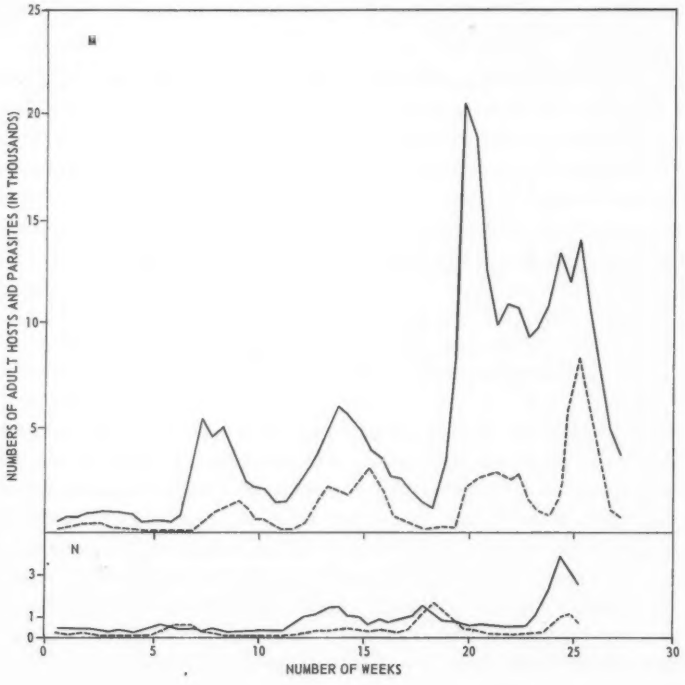


FIG. 5. Numbers of adult hosts and parasites counted in population M (infestation periods: host, short; parasite, short) and population N (infestation periods: host, long; parasite, long). Solid line, hosts. Dotted line, parasites.

(populations O and P) the second peak of the host was greater than the preceding one. Fifth, with long host introductions (populations N and O) peaks of parasite numbers were, in some cases, as high as corresponding peaks of host numbers, whereas with short host introductions (populations M and P) they were considerably lower.

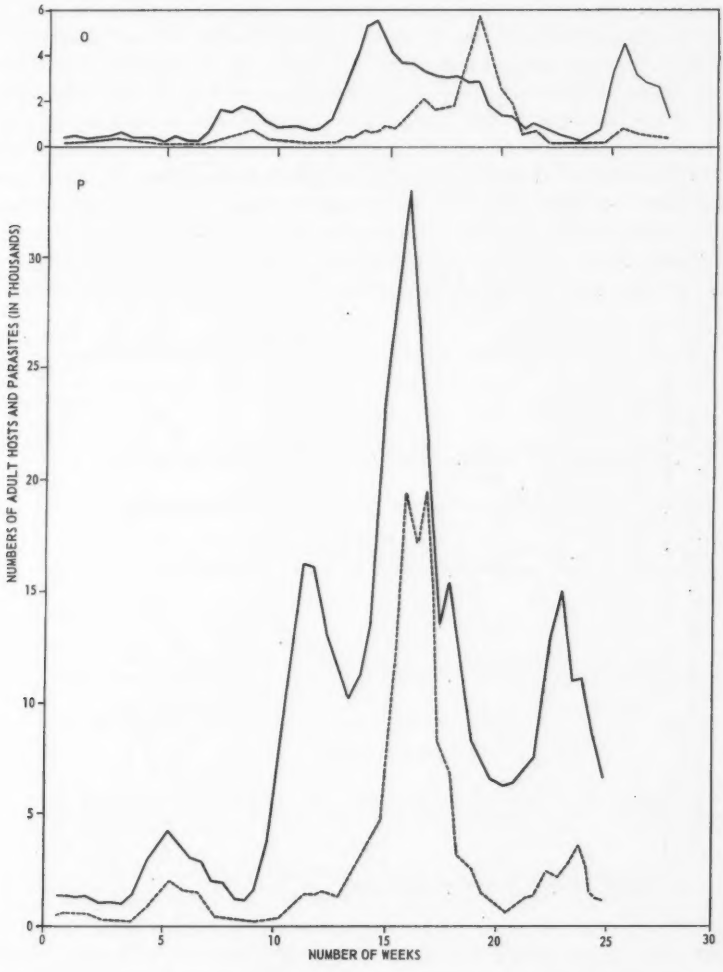


FIG. 6. Numbers of adult hosts and parasites counted in population O (infestation periods: host, long; parasite, short) and population P (infestation periods: host, short; parasite, long). Solid line, hosts. Dotted line, parasites.

*Distribution*

A comparison of the numbers of adult hosts and parasites recorded twice each week at each of the five points of sampling in each of the eight populations shows that the increase and decrease in the numbers of host and parasite adults tended to be synchronized throughout the population. For example, Fig. 7 shows that hosts and parasites in population N fluctuated with peaks of increasing magnitude at all five points. A possible exception to this trend occurred in population O, Fig. 8, where the last peaks of host numbers were not always greater than the previous ones; but it is possible—but not yet proved—that, in this case, the last peaks shown in Fig. 8 were only parts of complete fluctuations. While the numbers of host and of parasite tended to fluctuate in unison at each sampling point there were, of course, some differences in the numbers at each point. This variation is summarized, in an approximate manner, in Table III where the total number of hosts and the ratio of hosts to parasites for the first 15 weeks of each experiment are recorded. As the values in Table III can be altered by using different portions of the growth-forms of host and parasite, comparisons between populations are not too

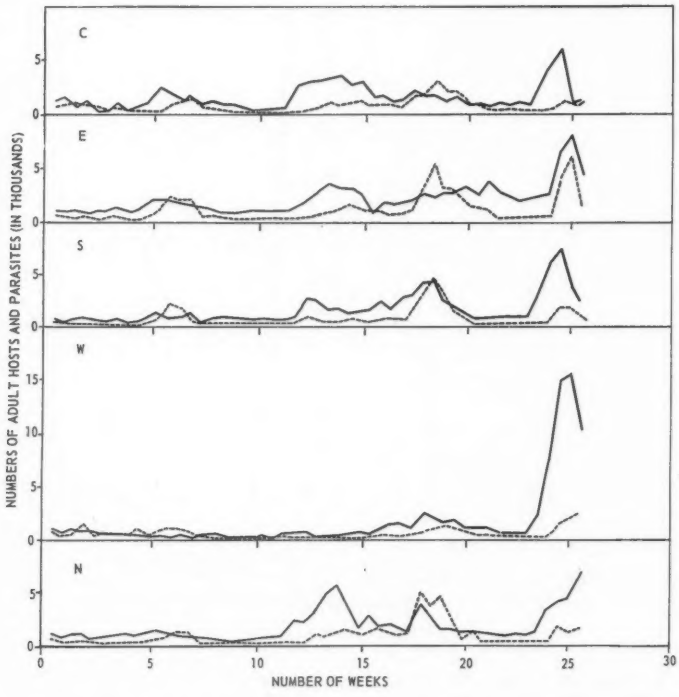


FIG. 7. Numbers of adult hosts and parasites counted in five areas of population N. C, center. E, east. S, south. W, west. N, north. Solid line, hosts. Dotted line, parasites.

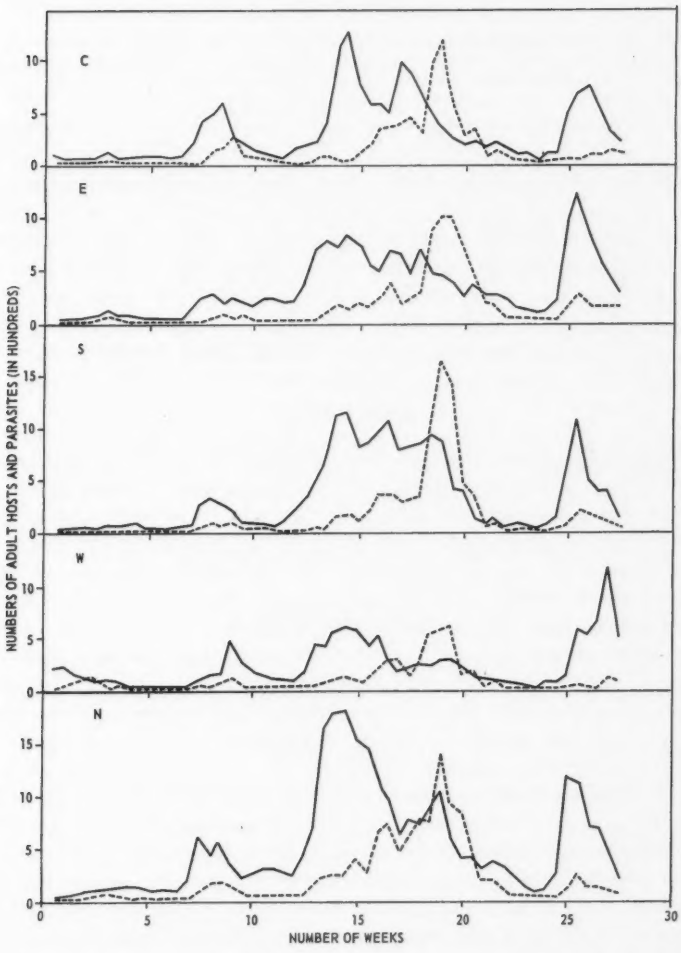


FIG. 8. Numbers of adult hosts and parasites counted in five areas of population O. C, center. E, east. S, south. W, west. N, north. Solid line, hosts. Dotted line, parasites.

meaningful. But Table III shows that both host and parasite occurred in all areas of the experimental units in substantial numbers and that the highest and lowest totals did not consistently occur in the same area of each population. Table III gives some evidence of the variation among the sampling points for the south and east points of population K and the north and east points of population N were contiguous while the east and center points in both populations J and P were diagonally adjacent. It appears, therefore, that there was as much variation between neighboring points as between more distant ones.

TABLE III

Total numbers (in thousands) of adult hosts and ratio of hosts to parasites recorded during first 15 weeks at five sampling points of eight populations of *T. vaporariorum* and *E. formosa*

| | Sampling point | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | North | | West | | South | | East | | Center | |
| Population | Host | Ratio | Host | Ratio | Host | Ratio | Host | Ratio | Host | Ratio |
| I | 28 | 3.5 | 28 | 3.9 | 23 | 2.9 | 36 | 4.5 | 24 | 3.9 |
| J | 60 | 4.7 | 61 | 5.3 | 54 | 4.6 | 41 | 4.2 | 59 | 4.3 |
| K | 59 | 4.5 | 33 | 3.0 | 58 | 4.4 | 68 | 4.6 | 49 | 4.0 |
| L | 76 | 3.7 | 69 | 3.7 | 74 | 4.0 | 78 | 4.7 | 60 | 3.6 |
| M | 14 | 3.7 | 12 | 3.0 | 12 | 3.3 | 19 | 4.0 | 15 | 3.0 |
| N | 4 | 3.1 | 0.9 | 1.0 | 3 | 2.1 | 4 | 2.6 | 4 | 2.6 |
| O | 13 | 5.1 | 6 | 4.3 | 7 | 6.1 | 7 | 5.7 | 8 | 5.1 |
| P | 37 | 6.4 | 30 | 6.2 | 37 | 6.6 | 35 | 5.7 | 36 | 4.8 |

## Conclusions

In the experiments described above four populations of *T. vaporariorum* and *E. formosa* were reared for 8 months in adjacent sections of a greenhouse, and a year later a second group of four were propagated similarly. During these extended periods there may have been slight differences in the average temperatures of the experimental units and the reproductive rate of the host may have varied slightly because of imperceptible changes in the quality of the host plants. But the populations of *T. vaporariorum* were always capable of very rapid growth and yet they were always subject to limitation by the parasite. In populations with exceptionally large fluctuations limitation of the growth of the host and parasite populations was imposed by the carrying capacity of the host plants but these restrictions did not occur until at least the third peak of host numbers was reached, i.e. until after several generations. When the conditions of the experiments were identical (populations J and K) the resulting growth-forms of host and of parasite populations were remarkably similar to each other. As the physical environment was relatively stable, the host population was usually restricted by the parasite population, and as the growth-forms of host and parasite were reproducible, the variation in the numbers of adult hosts and parasites resulted almost entirely from the interaction of the host and parasite populations.

In all eight experiments the numbers of adult hosts and parasites fluctuated with peaks of increasing magnitude in the initial portions of each growth-form. But the magnitude of the fluctuations appeared to be determined by the manner in which the experiments were started. With equal periods of infestation, the higher the initial densities of host and parasite the higher the resultant fluctuations. With equal initial host and equal initial parasite densities, long periods of host infestation resulted in relatively small subsequent fluctuations of host and parasite whereas short periods caused relatively high fluctuations. In these experiments and in previous ones (1) the peaks of parasite numbers were as high as or higher than corresponding peaks of host numbers if the period of infestation of the host was long and much lower if

the period was short. As the result of host–parasite interaction was so closely related to initial densities and periods of infestation of host and parasite it is apparent that, once started, the interaction was governed by systematic rather than by random factors. And, as the fluctuations occurred at many different densities it is clear that factors other than the influence of host and parasite densities on the rate of increase of host and parasite populations played a significant role in the interaction of *T. vaporariorum* and *E. formosa*.

Figures 1–6 show that many different growth-forms of *T. vaporariorum* and *E. formosa* populations occur. In making an analysis of the interaction of these two species, therefore, the initial phase of experiments with these populations must be controlled. An indication of effects that may be caused by slight differences at the beginning of experiments is seen in a comparison of the growth-forms of populations I and M (Figs. 1 and 5); while both had approximately equal periods of infestation, the former was begun with 1500 hosts and 1000 parasites whereas the latter was started with 1250 hosts and 900 parasites. It may be that, with such a great rate of increase of the host species, synchronization of host and parasite populations is particularly important. But with proper synchronization and initial densities, populations of *T. vaporariorum* and *E. formosa* may be propagated for sufficiently long periods at low and moderate densities to establish the nature of host–parasite interaction in these species.

## Acknowledgments

The assistance of Mr. G. D. Williamson is gratefully acknowledged. The figures were prepared by the bio-Graphic Unit, Department of Agriculture.

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# EFFECTS OF EXPERIMENTALLY ALTERED UNIPOLAR AIR-ION DENSITY UPON THE AMOUNT OF ACTIVITY OF THE BLOWFLY, CALLIPHORA VICINA R.D.[1]

D. K. EDWARDS[2]

## Abstract

Populations of the blowfly, *Calliphora vicina* R.D., were exposed to different densities of artificially produced unipolar air-ions. The activity of the flies was continuously recorded. Increased positive-ion densities resulted in increased flight activity. With a moderately high positive-ion density the peak activity occurred approximately 0.75 hours after first exposure to the ions. Following the peak, the activity returned to the original level while the insects were still exposed to the ions. No activity effect was observed with the negative-ion density level used.

## Introduction

Modifications in the behavior of many insects have often been observed before storms, and particularly before thunderstorms. There have been some suggestions in the literature as to the possible reasons for changes in insect behavior prior to storms. Most are speculative. Little experimental work has been done with a view toward controlling as many of the environmental variables as possible or maintaining the experimental limits close to those found in nature.

The possible role of atmospheric electricity as a modifier of insect behavior prior to, and during, certain types of frontal passage has been mentioned by Fabre (10), by Uvarov (22), and lately by Wellington (27). However, to the present time, insects have not been experimentally subjected to changes in two principal physical factors of atmospheric electricity, namely potential gradient and air-ion density, with a view to studying the effects, if any, upon insect behavior. The present studies were initiated to determine experimentally, first of all, whether changes in a potential gradient and in air-ion density could produce an effect upon the amount of activity of insects. If an effect was found it would then be necessary to determine whether the magnitude of the physical factor necessary to produce a change in the laboratory was a realistic one in terms of what would be expected to occur in nature.

The results of some experiments with the first factor, potential gradient, have already been reported (8). This study showed that, when there was an effect, activity in *Drosophila* and *Calliphora* was depressed following a change in potential gradient. The present paper deals with the results of a study to determine whether the amount of activity of *Calliphora vicina* R. D. may be modified by changes in the density of artificially applied unipolar air ions of both signs.

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*Nature of Air Ions*

Small ions, or drifting, charged particles, are produced in normal, free air by a variety of factors; these include cosmic radiation, electrical point-discharges, radioactive elements in the soil, and atomization of water (13). During periods of depression in barometric pressure, air is withdrawn from the soil along with amounts of the radioactive gases radon and thoron. The latter result in increased production of air ions (5). The ion-producing effects of soil emanations also can be related to diurnal changes in convection (16). The size spectrum of atmospheric ions runs from fast-moving, small, molecular complexes with a single electrical charge (mobility 0.12–2.0 cm/sec/v/cm) to slow-moving, large clusters of single charges attached to condensation (Aitken) nuclei (mobility 0.0005–0.12 cm/sec/v/cm). The latter are frequently found near industrial and urban centers. For the present purposes only the ions of small mass will be considered; the electrical charge on the large (Langevin) ion is relatively small and unimportant in relation to the mass of the ion, and the mobility is generally only about 1/500 that of the small ion (6). If there is an excess of ions of one electrical sign over oppositely charged ions, there is present a condition referred to as a "space charge". Small atmospheric ions have a lifetime of about one minute (25), but their continuous production can maintain the ion density.

*Past Work with Ions*

For a number of decades ionization of the air has been suspected as being a significant factor causing various biological phenomena (7). The question has been controversial, primarily because the emphasis has been on clinical methods of investigation which could not be adequately controlled. Further, earlier methods for producing artificially ionized air introduced additional variables, such as ozone and various oxides, into the experiments (2). Only recently has there been a trend toward a more fruitful approach. Laboratory animals are being used for experiments wherein a greater degree of control can be applied. The availability now of small, efficient ion-generators (14) has greatly facilitated experimentation.

Schilling and Holzer (19) have emphasized the need for studies of the possible relation between air electricity and physiological phenomena. Chalmers (6) mentions the fact that unperceived outside weather sometimes appears to influence the well-being of patients in closed hospital rooms. Wangermez (26) believed that some maladies peculiar to certain locales may be related to microclimatic electrical phenomena. It is not the purpose of the present work to enter into a review of the clinical aspects of past research into air-ion effects. The interested reader is advised to see Kornblueh (12) and Kornblueh and Griffin (13) for good summaries and bibliographies of the work done to date in this field. A comprehensive bibliography of Russian work in the field of therapeutic applications, which dates back several decades, is given by Vasilyev (23).

At present, the only published records of which the author is aware regarding effects of air ions on small organisms (excluding tissue cells) are

those of Krueger *et al.* (for example see ref. 14). There has been no published record to date concerning ion effects on insects (Vasilyev states (24) that there are two unpublished Russian works on this subject).

Beckett (2) believes that certain facts have already been established regarding the general biological effects of air ions. He concludes that although it is necessary to use extremely sensitive methods for testing, atmospheric ions occurring at relatively low densities in nature do have an effect, positive ions generally producing the most striking results. There is some evidence that a sudden change in the polarity of ionization is especially effective in producing some kinds of biological reactions in the laboratory (2, 13). In view of such statements, it seemed desirable to determine whether or not air ions affect insects, particularly when an attempt is made to keep the experimental density limits not far from those to be expected in nature. Since there is evidence that different air-ion densities also are associated with different kinds of weather (15, 17, 18), the possibility arises that insect behavior sometimes may be influenced by weather through the medium of unipolar air ions as well as by other physical variables.

## Methods and Procedure

*Methods*

Adult blowflies (*Calliphora vicina* R.D.) were used in the experiments. They were reared in the laboratory on a specially prepared culture medium at 23°–25° C. The adult insects were caged in the laboratory and fed sucrose crystals and, occasionally, raw liver.

The method which was developed for obtaining a continuous record of the amount of activity occurring in the experimental groups of insects has been described in another paper (9). The insects to be tested were put into a Faraday cage enclosed in a steel cabinet (Fig. 1). Both boxes were earthed. A Keithley model-6101 shielded test-lead (Fig. 1, *e*) and probe (*f*) projected through the sides of the two boxes, so that the tip of the probe was in the center of the inner cage. Care was taken to ensure that the sensitive probe was highly insulated from contact with any part of the box. The shielded test-lead running from the probe through the top of the cage and cabinet was attached to a Keithley model-600 electrometer; the output from the electrometer drove a Varian G-10 graphic recorder.

An insect flying within the inner box generates a positive charge on its body. This produces an electrical influence on the sensitive probe; the resulting sudden change in potential upon the probe is amplified by the electrometer and is recorded as a deflection from the base-line on the recorder chart. Thus, by counting the number of positive deflections occurring in a given time on the chart a continuous record is obtained of the amount of activity within the cage throughout the experimental period. A more detailed discussion of the interpretation of the chart record is presented in reference 9.

For the experimental introduction of unipolar air ions into the insect cage, a small, radioactive-type (tritium) ion generator (Fig. 1, *g*) made by the

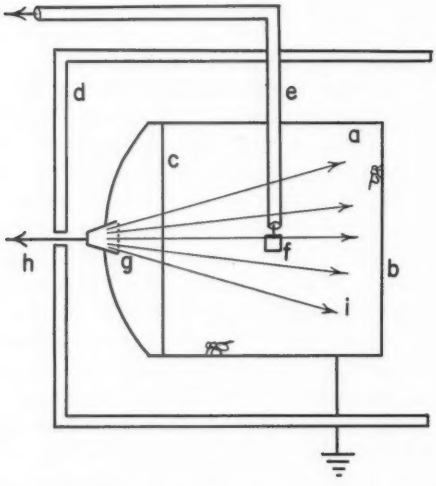


FIG. 1. Apparatus for recording flight activity in blowflies: (*a*) insect cage, or Faraday cage; (*b*) copper screen; (*c*) plastic screen; (*d*) metal cabinet; (*e*) shielded test-lead (to electrometer); (*f*) probe; (*g*) ion source; (*h*) connection to voltage source; (*i*) ions.

Wesix Electric Heater Co. was fastened with a plastic holder behind the inner cage, opposite its plastic screen. With a potential applied between the ion source and earth, unipolar air ions were carried by the electrical field through the plastic screen into the insect cage. By varying the magnitude and polarity of the potential on the ion source, the density and electrical sign of the ions could be altered. The electrometer probe, in the center of the insect cage, was located about 10 inches in front of the ion source and was separated from it by the plastic window-screen. The probe monitored the ion current at the cage center; with the ion source "on", a steady deflection was produced on the chart which was proportional to the amount of ion current to earth in the middle of the cage; in addition, the probe responded to deflections caused by insect flights. Measurements of the net number of ions striking the probe at the center of the cage were obtained with a Beckett probe (14). This method did not give an absolute indication of the ion density at the center, but only the magnitude of the net ion current to earth through the probe caused by a surplus of ions of one polarity over oppositely charged ions. Indeed, it would have been difficult, if not impossible, to measure the absolute numbers of ions of each sign within such a confined space as the insect box. Methods for making this type of measurement require a large volume of air and would affect the numbers of ions present. At points within the insect cage nearer the ion source than the probe, the ion density during the experimental period naturally would have been greater than the measured amount; similarly, the density would have been less than the measured amount at points farther from the ion source than the probe.

With a *negative* potential on the ion source high enough to produce a steady negative deflection of the base-line on the recorded chart of about 1 cm (i.e. about −345 v) it was not possible to obtain a reliable estimate of the number of insect flights by examining the chart. The explanation for this follows. With −345 v on the ion source the base-line on the chart was maintained on the negative side of the neutral point by the constant flow of negative ions. However, each time an insect walked across the screened surface of the box nearest the ion source (Fig. 1, *c*), between the source and the probe, a "shadow effect" was produced. This "ion shadow", cast by the insect on the probe, resulted in return of the recorder pen to the neutral base-line level, followed in a few seconds, as the insect moved away, by a resumption of the original level of steady, negative deflection. The result of this "shadowing" was a series of deflections on the *positive side* of the negatively deflected base-line, with the longest deflection equalling the amount of steady negative deflection of the base-line (i.e. 1 cm). When examining the record later, it was difficult to separate positive deflections caused by flights from positive deflections caused by "shadowing". At the lower experimental potential of 115 v the steady, negative deflection of the base-line was very small, and the positive deflections due to "shadowing" were smaller than the 3-mm threshold value set for counting the deflections. Of course, this "shadowing" problem did not arise at all when using positive potentials on the ion source. In this case, the steady deflection of the base-line due to ion flow was in a positive direction, with "ion shadows" being *negative* deflections (returning to the neutral level). Since the insects are positively charged when flying, only positive deflections were counted at all times.

*Procedure*

The groups of experimental insects were introduced into the insect cage without etherization at least a day prior to the beginning of the experiments. The insects were fed daily with sucrose solution, and were sometimes maintained in the cage for as long as 2 weeks in good condition. A constant source of illumination was fixed above and in front of the cage. In all experiments the electrometer voltage-range was set at 0–0.03 v, and the electrometer shunt at $10^9$ ohms. The shunt prevented a charge from accumulating on the probe. The recorder span was set at 100 mv full scale, and the chart speed was 2 inches per hour.

In the first series of experiments, a potential of +345 v was applied to the ion source. The series was divided into two parts. One group of five male and five female *Calliphora* was put into the insect cage on February 7, with experiments on February 8, 9, and 10. Another group, with the same number of insects, was put into the apparatus on February 24, with experiments on February 25 and 26.

On the days when experiments were carried out, the chart record of activity in the cage was begun at $1000^h$ and finished at $1615^h$. From $1000^h$ to $1100^h$ there was no increased ion density. The ion source was turned on at $1100^h$ and off at $1230^h$; a second period of increased ion density was run from $1345^h$

to 1515$^{h}$. Thus, the day-long experiment could be broken into two separate experimental runs: in both the morning and afternoon there was a 1.5-hour period of increased ion density; each period of increased ion density was preceded, and followed, by a 1-hour control period, during which there was no artificially increased ion density. The morning second control-period overlapped the afternoon first control-period by 45 minutes. A total of 10 experiments with +345 v, each consisting of a control 1, experimental, and control 2 period, was carried out.

A series of experiments was performed using a potential of +115 v on the ion source. One group of five male and five female *Calliphora* was used between February 11 and February 24. Another group was studied between February 29 and March 4. Two experiments were carried out on each day, in the manner indicated above, to give a total of 22 experiments with +115 v.

A series of experiments was carried out with a negative potential of 115 v on the ion source. In these, one group of five male and five female *Calliphora* was studied over a period of 12 days, to give a total of 22 experiments with negative ions.

## Results

*Ion Source +345 v*

The pen-recorder tracing from one experiment, using a potential of +345 v on the ion source, is shown in Fig. 2. The 1.5-hour period of increased positive-ion density is indicated by the center portion of the tracing; this part was displaced to the positive side of the neutral base-line by the positive ions.

In all 10 chart records, the number of positive deflections in each 7.5-minute interval which exceeded 5 mm in length was counted. The mean was found for all the corresponding single-interval sums in the 10 experiments, using the start of the experimental ion-period as a point of reference in each

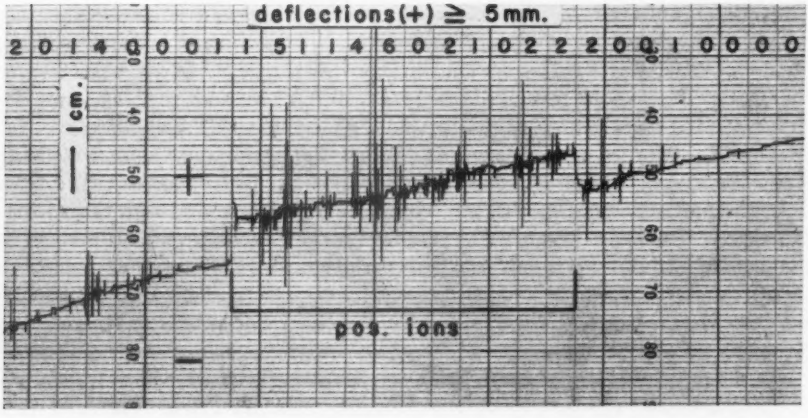


FIG. 2. Tracing from an experiment with +345 v on ion source. Numbers of positive (upper) deflections, exceeding height 5 mm, in each 7.5-minute chart interval, are indicated.

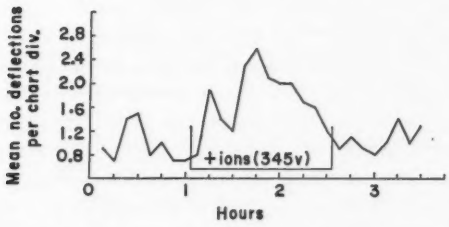


FIG. 3. Effect of 345-v positive ions on mean number of deflections per chart interval, for 10 experiments.

case. The mean numbers of spikes, exceeding 5 mm, in each chart division in 10 experiments are plotted against time in Fig. 3.

The 1.5-hour experimental period of increased positive-ion density, and the 1-hour control periods at both ends of the experiment are indicated in Fig. 3. It is plain that the period of increased positive-ion density resulted in an initial rise in the number of deflections on the records (i.e. number of flights); this was followed by a decline in activity to a level approximately equal to that prior to application of the ions. The mean peak activity was reached about 0.75 hours after first application of the ions. The later decline in activity during the experimental period indicates acclimatization of the flies to the increased number of ions. There was no "undershoot" in the activity level following elimination of the increased ion-density.

Measurements with the target-collector, or Beckett-probe, method (14) of the normal, "background" space-charge present in the cage during mid-afternoon gave a value of 127 excess positive ions/mm²/second striking both sides of the probe in the cage. This resulted in an ion current through the probe of $2.04\times10^{-15}$ amp/cm². This net ion flow to earth was taken as the control value for these experiments. Application of a positive potential of 345 v to the ion source resulted in an ion flow of 3560 positive ions/mm²/second, or an increase of 3433 ions/mm²/second over the background value (increased current $5.2\times10^{-14}$ amp/cm²).

The mean numbers of deflections in each of the three parts of the 10 experiments are given in Table I. The table also shows the mean differences between: experimental period minus control 1; experimental minus control 2; and control 1 minus control 2. A paired-*t* test was performed on the three sets of differences (Table II). The *t* test showed that the experimental-period means were significantly higher than the control 1 means, at the 5% level; the experimental means were higher than control 2 at the 5% level; there was no significant difference between control 1 and control 2 means.

If there was a trend toward a higher level of activity in any one of the experimental or control periods compared with the remaining periods, mean differences between the periods would show a greater number of values of one sign compared with the opposite sign. The number of positive values in each category of differences was compared with the number of negative

TABLE I

Mean numbers of deflections in each of the three parts of 10 experiments, with days in apparatus; paired differences are indicated (E = experimental period; C1 = control 1; C2 = control 2); ion source +345 v

| Days | Control 1 (C1) | Experimental (E) | Control 2 (C2) | Paired differences | | |
|---|---|---|---|---|---|---|
| | | | | E−C1 | E−C2 | C1−C2 |
| 1 | 1.62 | 0.66 | 0.62 | −0.96 | 0.04 | 1.00 |
| 1 | 0.75 | 2.83 | — | 2.08 | — | — |
| 2 | 0.87 | 2.75 | 1.87 | 1.88 | 0.88 | −1.00 |
| 2 | 1.25 | 1.83 | 1.50 | 0.58 | 0.33 | −0.25 |
| 3 | 0.12 | 0.83 | 1.00 | 0.71 | −0.17 | −0.88 |
| 3 | 0.87 | 1.08 | 0.87 | 0.21 | 0.21 | 0.00 |
| 1*a* | 1.50 | 1.66 | 0.87 | 0.16 | 0.79 | 0.63 |
| 1*a* | 1.37 | 1.91 | 0.87 | 0.54 | 1.04 | 0.50 |
| 2*a* | 1.00 | 2.08 | 0.37 | 1.08 | 1.71 | 0.63 |
| 2*a* | 0.25 | 1.66 | 1.25 | 1.41 | 0.41 | −1.00 |

TABLE II

Means of experimental period (E) minus control 1 (C1), experimental minus control 2 (C2), and control 1 minus control 2 differences, in all experiments

| Source voltage | Ion charge | No. expts. | Means of differences | | |
|---|---|---|---|---|---|
| | | | E−C1 | E−C2 | C1−C2 |
| 345 | + | 10 | 0.77* | 0.58* | −0.04 |
| 115 | + | 22 | 0.65* | 0.37 | −0.21 |
| 115 | − | 22 | 0.14 | −0.13 | −0.06 |

*Significant at 5% level.

TABLE III

Numbers of positive and negative values in each category of mean differences, with chi² value for category; E = experimental period, C1 = control 1, C2 = control 2

| Source voltage | Ion charge | Difference category | Number of values | | Chi² |
|---|---|---|---|---|---|
| | | | + | − | |
| 345 | + | E−C1 | 9 | 1 | 6.4* |
| | | E−C2 | 8 | 1 | 5.4* |
| | | C1−C2 | 5 | 4 | 0.1 |
| 115 | + | E−C1 | 16 | 6 | 4.5* |
| | | E−C2 | 15 | 7 | 2.9 |
| | | C1−C2 | 8 | 12 | 0.4 |
| 115 | − | E−C1 | 14 | 8 | 1.6 |
| | | E−C2 | 12 | 9 | 0.4 |
| | | C1−C2 | 12 | 9 | 0.4 |

*Significant at 5% level.

values by means of a chi² test (Table III). The test for a 1:1 ratio in the number of positive and negative values showed that there were significantly more positive than negative values in experimental-minus-control-1, and in experimental-minus-control-2 differences. There was statistically the same number of positive and negative values in the control-1-minus-control-2 differences, indicating no tendency for one control level to be higher than the other.

The tests outlined above show that the mean activity was greater in the experimental ion-period compared with the control 1 and control 2 periods. There was no difference in activity level between control 1 and control 2 periods.

*Ion Source +115 v*

A target-collector determination, with a potential of +115 v on the ion source, showed the total number of positive ions striking the probe to be 382 ions/mm²/second (255 ions/mm²/second over the background number). This resulted in a total ion current to earth of $6.1\times10^{-15}$ amp/cm² (increase of $4.1\times10^{-15}$ amp/cm²).

The same procedure was used for analyzing the activity record as was used with the 345-v data, except that the spike-height threshold was 3 mm instead of 5 mm. Means were obtained for the number of deflections in the control 1, experimental, and control 2 periods in 22 experiments. Mean differences were obtained between experimental and control periods. Table II indicates the means of the differences and whether or not they are statistically significant. A paired-*t* test on the differences showed that experimental-period means were higher than those of control 1 at the 5% level. There was no significant difference between experimental and control 2 means, or between control 1 and control 2 means.

A chi² test for a 1:1 ratio in the numbers of positive to negative values in the sets of mean differences gave the values in Table III. The results of this test are in agreement with the results obtained in the paired-*t* test; there were significantly more positive than negative values in the experimental-minus-control-1 differences, and there were no significant differences in number of positive compared with negative values in experimental-minus-control-2, and in control-1-minus-control-2 categories.

The effect on activity of the 115-v positive ions does not seem to have been as great as that found with a potential of 345 v. In the latter, only 10 experiments showed an effect which was significant at the 5% level; in the +115-v series, 22 experiments were necessary to show an effect significant at the 5% level.

*Ion Source −115 v*

Measurements with a Beckett probe showed that the net number of negative ions reaching the target was 382 ions/mm²/second.

The analysis of the data was identical with that for the positive ions of 115 v. Table II gives the means of the three categories of differences for 22 experiments. A paired-*t* test showed no significant differences in any of the

paired categories. The ratio of numbers of positive to negative values in each of the three difference-categories was tested with $chi^2$; Table III shows that there were no differences between the numbers of positive and negative values, and hence that the activity levels throughout tended not to differ.

In addition to this series of 22 experiments with one group of insects, 8 more experiments were later carried out using different individuals. The experimental-minus-control-1 differences gave five negative and three positive values. This brings the total E−C1 differences (Table III) to 17 positive and 13 negative values; this is even closer to a 1:1 ratio.

## Discussion

The experiments reported here have demonstrated that an increased positive air-ion density can influence the amount of flight activity in the blowfly, *Calliphora*. The changed activity was expressed as an increase in the amount of flight activity during the period of artificial ionization. There is evidence that the insects are able to acclimatize to increased positive-ion density, after a period of about 1-hour exposure. No activity effect could be shown with negative ions, with an ion-source potential of 115 v. Although the possible effects of a higher density of negative ions were not investigated for reasons already mentioned, there is good reason to believe that if negative ions can influence the activity of *Calliphora* the effect is not as definite as that produced with positive ions.

It can legitimately be asked, at this point, whether the radioactivity of the tritium source, or whether the potential gradient from the potential on this source, themselves could have influenced the activity of the insects. Tritium is a soft-beta emitter with an insignificant amount of secondary radiation; a typical Bremsstrahlung measurement 16.5 cm from the ion source (14) showed only 0.8 mr/hour. Further, the insects were constantly exposed to what little radiation there may have been from the source, not only during the experimental period. The potential gradient used in the present experiments (maximum of 345 v acting over a distance of about 25 cm to the center of the cage) was insufficient to produce an activity effect in *Calliphora*. Edwards (8) has reported experiments showing that a field effect on *Calliphora* activity required a potential gradient higher than 300 v/cm; even then the effect was in the direction of a very brief ***reduction*** in activity immediately after application of the field, followed by a return to the original activity level. A potential on the ion source caused no change in temperature.

In the present experiments it was not possible to obtain a volumetric measure of absolute ion-densities within the experimental Faraday cage. The volume of air required for this type of measurement is too large. Most reports of ion densities during different weather conditions give data in terms of ions, or e.s.u., per unit volume of air. In order to obtain field data which can be related to data resulting from the present experiments, studies should be undertaken in the field using the same laboratory apparatus to obtain relative values. Of course, space-charge (excess number of ions of one sign)

measurements, such as those made with the Beckett probe, would not provide information regarding the absolute numbers of ions present.

Most authors agree that under normal conditions of fair weather the space charge outdoors is not high. Skilling and Beckett (21) report that small positive ions generally exceed small negative ions in a ratio of 5:4, resulting in a small positive space charge. Wangermez (26), studying the ratio of positive to negative ions, found no great difference in the ratio between data obtained on a beach and in a nearby forest. The ratio in the forest (positive: negative) was 0.6, 1.3, and 1.0. The air–earth current can tell us something about the space charge. Bartels (1) gives a value for air–earth current, measured directly, of $3\times10^{-16}$ amp/cm$^2$. Indoors, one frequently finds a higher, positive, space charge compared with that outdoors, resulting in a slightly higher current flow from earth (e.g. the "background" value in the present experiments was $2.04\times10^{-15}$ amp/cm$^2$).

During periods of turbulent or unsettled weather the ion density near the earth's surface, and the space charge, may change. Discharge currents from projections from the earth may cause regions of positive space charge when influenced by the charged base of thunder clouds (20). When point discharge is occurring, ions are liberated and travel downwind from the point, often producing violent variations in space charge to leeward of the point (6). Bartels (1) states that "during squalls and thunderstorms, the (air–earth) current flows upward or downward, and the point discharges over grass can furnish current densities $10^5$ times as high (as the normal value, $3\times10^{-16}$ amp/cm$^2$). . .". This would give a value $3\times10^{-11}$ amp/cm$^2$. The present experiments have shown that an increase in the positive-ion current of about $5.2\times10^{-14}$ amp/cm$^2$ over the "background" value was accompanied by a clear change in the activity of *Calliphora*. Thus, the magnitude of ion current to earth measured in the center of the insect cage in these experiments might be expected to be found in nature. The question, however, which cannot be answered at the moment, is whether the activity of the insects is influenced by the air–earth current or by the air ions per se (e.g. positive ions). In these experiments, the former was within natural limits, while the latter, positive-ion density, was somewhat higher than that expected in nature.

Norinder and Siksna (18) have shown that during certain types of rain showers a rise occurs in the concentration of the small ions of both polarities, the increase in negative ions being faster than that of positive ions. One case is reported of a surplus negative-ion density of 5000 I/cc. No influence was observed by Norinder and Siksna of direct lightning strokes near the observation point on the ion density. These authors also described changes in the density of ions in the vicinity of a thunderstorm. A decrease in the negative-ion density for 15–20 minutes was observed. At the same time the positive-ion density, which had also decreased at the same time, elevated to normal more quickly.

Other factors may cause increases in air-ion density and space charge. A wind blowing off the land may have a higher ion density than one from the

sea; a change of air mass can result in a changed ion density (17). The föhn winds in Europe have been reported to have a greater than usual excess of positive ions (3). Smoke caused by forest fires can result in very large positive space-charge values (4); this might be an important factor in relation to forest-insect behavior.

In considering the effects of weather on the behavior of some organisms located within buildings, such as experimental insect populations, it is important to take into account the ion densities within the buildings, and the changes in ion density which occur with different kinds of weather. Yaglou *et al.* (28) and Kähler (11) found the ion content of air in buildings not too different from that outdoors. These authors reported that the ion content decreased in occupied (peopled) rooms compared with that in unoccupied rooms. Beckett (3) states that the ion density in a room depends much upon the type of ventilation; in a sealed room it is lower than that outdoors, window ventilation equalizes the ion densities indoors with those outdoors, and forced-air through long ducts tends to increase the positive ion excess by reducing the number of negative ions.

It is apparent from the work done in the past, and from the results of the present experiments, that air ions can produce a biological effect. They can influence the amount of activity in blowflies. Further, it is known that the density of air ions under natural conditions can vary considerably with different types of weather. There is a need for more studies to determine: whether insects respond to the air ion density in the space around them, or to the air–earth current, or both, when they are at rest; whether the magnitude of air-ion density, or air–earth current, is sufficient in the field under certain conditions for these factors to play a significant biological role in relation to other environmental parameters; the mechanism of action, and effects, if any, of long-term exposure of insects to ions (particularly positive ions) on fecundity and vitality.

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# THE SUBSPIRACULAR GLANDS OF HYALOPHORA CECROPIA (L.) (LEPIDOPTERA:SATURNIDAE): HISTOLOGY AND CYTOLOGY[1]

R. HARMSEN[2] AND W. E. BECKEL

## Abstract

A histological and cytological study of the subspiracular glands of the giant silkworm *Hyalophora cecropia* is reported. These glands are true organs, situated in the first eight abdominal segments, one gland under each spiracle. The glandular tissue is made up of extremely large cells varying from 15 to 25 $\mu$ by 8 to 18 $\mu$ in the first instar, and from 150 to 600 $\mu$ by 60 to 150 $\mu$ in the fifth instar and pupa. A cellular sheath encloses the glands in the later instars. Many tracheoles penetrate this sheath and disperse among the glandular tissue.

The glands appear to secrete before each larval molt, before and after pupation and after the breaking of diapause in the pharate imago. After the last secretion the cells break down. All secretions are preceded by an enlargement of the nucleus in the earlier instars and by a profuse branching of the nucleus in the later instars. A nucleolus is observed prior to, and during each secretion, except for the secretions before and after pupation. The secretion appears to begin in the nucleus and extend through the cytoplasm to the blood. In the later larval instars, the gland cells increase in number by means of amitotic division.

A comparison of this study with the literature makes it possible to conclude that in all holometabolous insects, a glandular structure can be found which is homologous to the subspiracular gland of Lepidoptera.

A brief description of the subepidermal glands of *Hyalophora* is given to illustrate the distinction between the subepidermal gland cells which are often given the name oenocytes and the subspiracular gland cells which also have been called oenocytes.

## Introduction

In 1856 Fabre (7), describing the fat body of Sphegidae larvae (Hymenoptera), found, in addition to the normal cell type, large opaque cells that had been produced under the skin and had travelled inwards to disperse among the fat body. Similar structures were described for *Bombyx mori* (Lepidoptera) in 1882 by Tikhomirov (29). He considered the cells to be glands, described their location in the larva as a cluster of cells below the spiracles, and traced their origin as an outgrowth of the ectoderm. Carnoy, in 1885 (5), wrote about "yellow intercalated cells" in the fat body of most arthropods. He observed that these cells contained crystals. Furthermore, he described the increase in cell number as the result of amitotic division. In 1886 von Wielowiejski (34) described these cells for most insect orders. He studied them in detail in the larvae of *Chironomus* (Diptera) where they appear as large cells clustered in small groups under the spiracles. Von Wielowiejski named these cells oenocytes and supposed that they had an endocrine function.

It appears that Tikhomirov's "gland cells" in *Bombyx* and von Wielowiejski's "oenocytes" in *Chironomus* are homologous. However, the cells described by Fabre and Carnoy and also several "oenocytes" as described for other insects by von Wielowiejski, could be any type of abdominal endocrine glands.

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Verson and Bisson in 1891 (32) gave a detailed description of clusters of apparently glandular cells, lying ventral to the spiracles in the first eight abdominal segments of *Bombyx mori*. They found that these glands showed cyclic nuclear secretions throughout the life of the larva. They also reported that the number of cells per gland remained constant until they broke down in the pharate imago. They named the structures subspiracular glands. In the following year Verson (30) described another abdominal endocrine gland in *Bombyx*. The cells, in this case, were formed in the epidermis of the "spinning" larva and then migrated inwards in the pupa to form strands of glandular cells just under the ventral integument. They were observed to increase in number by amitotic divisions, to show secretory activity in the pupa and pharate imago, and to persist in the adult. Unfortunately Verson published in Italian; thus his work never became widely known, despite a short review article published in German in 1911 (31). In this latter communication he briefly described the various glandular structures of apparent endocrine function as found in *Bombyx*. The structures mentioned were:

(1) peritracheal and pericardial cells,
(2) prothoracic gland cell strings,
(3) subspiracular gland cell clusters,
(4) postlarval, subepidermal gland cells.

All these glandular structures showed a cyclic secretory activity (31) which seemed to be related to ecdysis. However, only for the prothoracic gland (possibly of cephalic origin) is a function known.

For the next 30 years much literature was published concerning the various abdominal endocrine glands; most often the glands were referred to as oenocytes. These investigations, however, were usually directed at only one developmental stage, as the gland cells were thought of as static units which did not vary with the insect's development. This approach may have caused the many contradictory reports found in the literature concerning location, cellular structure, color, and size of the gland cells. It seemed useful, therefore, to study the abdominal endocrine glands of one insect at a time and these glands at all developmental stages of that insect, assuming that the gland cells are not static but dynamic, and may change during the development of the insect.

A preliminary comparison of the subspiracular and the postlarval subepidermal glands of *Hyalophora cecropia* (L.) with those of other Lepidoptera described in the literature showed that *Hyalophora* could be considered representative of this order (2, 9, 11, 25, 27, 28, 29, 30, 32). Only minor differences were reported for *Bombyx* (29, 30, 32), *Ephestia* (27, 28), *Hyponomenta* (13), and *Vanessa* (11).

In a previous communication (10), the authors have reported the results of a histological study of the origin and intraovular development of the subspiracular glands of *Hyalophora*. The purpose of the present investigation is to follow the complete histological and cytological development of the subspiracular glands in the larva and pupa of *Hyalophora cecropia*. It would then

be possible to correlate the results with the scanty and often contradictory observations of other authors. Also a foundation would be laid for a study of the function of the glands.

## Materials and Methods

Fertilized eggs of the giant silkworm *Hyalophora cecropia* were kept until eclosion. The newly emerged larvae were reared on willow foliage. Sample specimens were taken for investigation throughout development up to and including the fully matured adult.

Specimens were fixed in either Carnoy's acetic alcohol (with and without chloroform), saturated sublimate, K.A.A.D. mixture, aqueous and alcoholic Bouin's, neutral formol, or Zenker's solution. Of these, the alcoholic Bouin's gave superior results. Consequently, this fixative was used for all the material to be described. For impregnating and embedding, paraffin wax with m.p. 60–63° C was used. Sections were cut at 5, 10, 15, and 20 $\mu$, depending on the size of the tissues. For small larvae, and small pieces of large larvae, pupae, and adults, the conventional sectioning technique was used. Where whole pupae, adults, or large larvae were sectioned, the tape method of transferring sections onto slides was employed (3). All sections were stained with Hansen's trioxyhaematin, an iron haematoxylin stain. Some observations were made on living materials, using a dissecting microscope and a phase contrast microscope.

## Histology of the Glands

At the moment of eclosion, each subspiracular gland consists of approximately 125 large glandular cells attached to each other and to surrounding tissues by filaments of what will ultimately become a continuous sheath around the gland.* Nuclei are sparsely found in the filaments. The glands are paired, and are found in each of the first eight abdominal segments immediately underneath the integument, ventral and slightly caudal to the spiracles. This situation persists (2, 9) (Fig. 1) until the final breakdown of the organs in the developing adult.

This glandular tissue, with its trachea and sheath tissue, develops into an intricate organ during the life of the larva. The development of the glands takes place only during the periods of general larval growth in the later instars. From 24–36 hours before the molt to shortly after the molt, no development of the glands is observed. During this period the gland cells show what appears to be secretory activity.

Throughout the growth of the first instar larva no observable change takes place in the general structure of the glands. In the growth periods of the second and third instar the number of gland cells increases, forming

*This sheath is apparently an extension of the so-called basement membrane which lies just under the epidermis and lines the haemocoel. There is disagreement as to whether this membrane is cellular or not. We have often observed nuclei in it (Fig. 8). If it is found to be cellular generally, even though the nuclei are sparse, it should not be called a basement membrane but rather an endothelium.

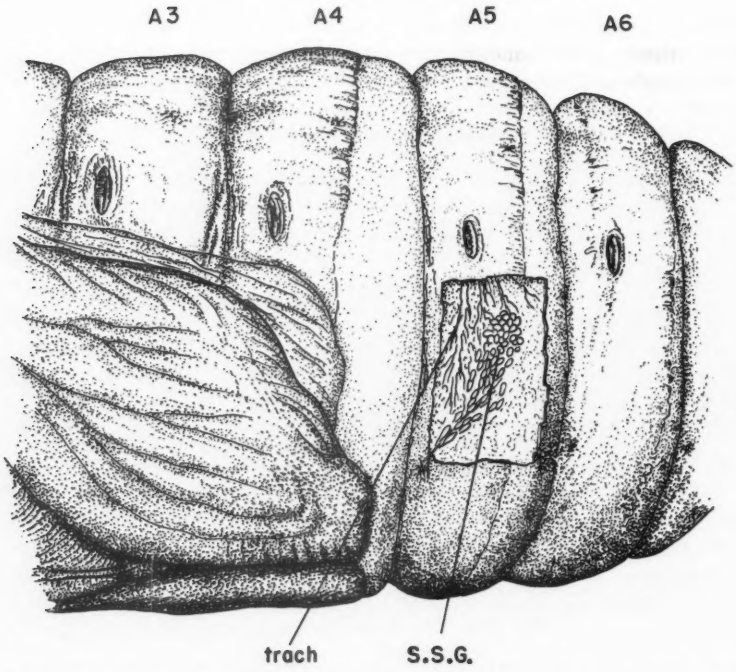



FIG. 1. Lateral view of a pupa of *Hyalophora cecropia* with a window cut in the fifth abdominal segment, showing the location of the subspiracular glands.

ABBREVIATIONS USED IN FIGURES:

$A_5$—fifth abdominal segment.
*bas. m.*—basement membrane.
*cyt. br.*—cytoplasmic bridge.
*ect*—ectoderm.
*epid*—epidermis.
*epid. ext.*—epidermal extensions.
*haem*—haemocyte.
*nuc*—nucleolus.
*rod. sp.*—rod-shaped space.
*shth*—sheath tissue.
*S.S.G.*—subspiracular gland.
*sub. epid. gl.*—subepidermal gland.
*trach*—tracheal tissue.
*vac*—secretion vacuole.

strands each consisting of two or three cells. The thin sheath filaments increase with the number and size of the gland cells and begin to envelop the growing glandular tissue (Fig. 3). However, it is not until the period of growth following the third larval molt that the glands develop into complete organs. This period, between the third and the fourth (last) larval molt, lasts from 8–10 days, varying with the individual.

Twenty-four hours after the third larval molt, there is no sign of cellular activity; yet 36 hours after, many of the glandular cells are in division, forming long strands of up to five cells (Fig. 10). The cells in the strands are still attached to one another by cytoplasmic bridges through which in some cases runs a thin thread of nuclear material. No evidence of mitosis is visible, but many cells in varying degrees of constriction are to be found. It is assumed, therefore, that the cells divide amitotically through constriction. The increase in number of gland cells is rapid. Three days after the third larval

molt no more constrictions can be found. All cells, nevertheless, retain their cytoplasmic bridges (Fig. 11). Thus, each gland is now composed of a cluster of approximately 160 cells, adhering to one another with cytoplasmic strands. These cells are interwoven with an extensive network of sheath tissue, which is penetrated by tracheoles.

Between 4 and 6 days after the third larval molt, the number of cytoplasmic bridges gradually decreases. During this period another feature of the development becomes apparent: the sheath and tracheal tissue increase very rapidly, so that by 6 days after the molt each gland cell is completely wrapped in tracheoles and sheath filaments. Similar filaments connect the cells to one another and to surrounding tissues. From 2 days before the last larval molt until approximately 2 days after, no development of the glands takes place.

In the fifth instar larva the same developmental procedure occurs. First the glandular tissue increases in cell number through what appears to be constriction division, then the tracheal and sheath tissues develop. In this final growing stage of the glands, in contrast to the earlier larval instars, the cytoplasmic bridges between all cells are retained. Also the growth of the tracheal and sheath tissues is more intense.

In the fully mature larva, each gland cell is enveloped in a cellular sac-like sheath in which scattered nuclei are observed. These sacs are attached to one another with tubular connections enclosing the cytoplasmic bridges that connect the gland cells. In this way, the sheath completely surrounds the gland cells (Fig. 4). The gland is attached to surrounding tissue by filaments of the sheath tissue. From each main ventral tracheal trunk a side branch forms the tracheal tissue of a gland. The trachea branches profusely penetrating the gland. The tracheoles are found attached to and penetrating the sheath (Figs. 4 and 5). It has been reported that tracheoles actually penetrate the gland cells (24). In *Hyalophora*, however, such a situation was not observed with certainty.

The above described gland remains unchanged throughout the life of the spinning larva and the diapausing pupa. After imaginal development has started the glands break down. First the sheath disappears, and the tracheal tissue is retracted. Immediately afterwards the gland cells show large vacuoles, and the cell membranes and nuclei disintegrate (Fig. 7B).

## The Cytology of the Gland Cells

### *1. The Secretion*

Shortly before eclosion, before each larval molt, at pupation, and immediately after the breaking of the pupal diapause, the glands show evidence of activity which we interpret as a secretion. In between the periods of secretory activity the gland cells divide and increase in size. The intraovular secretion cycle has been described in an earlier paper (10).

At the moment of eclosion, the gland cells are oval or slightly angular where touching one another (Fig. 2A). They are among the largest cells in

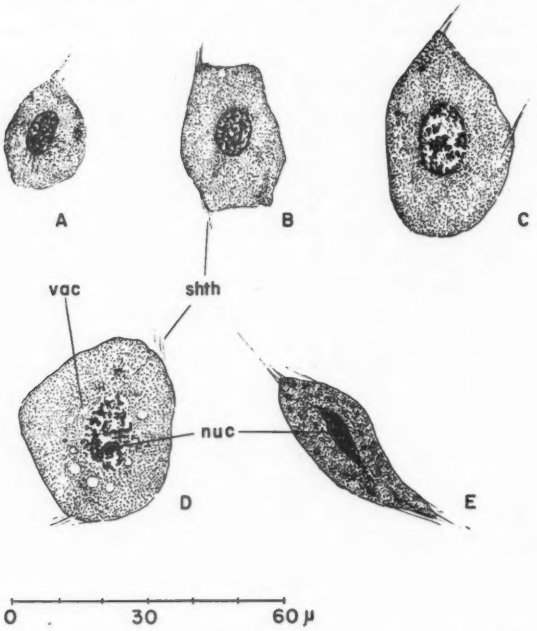
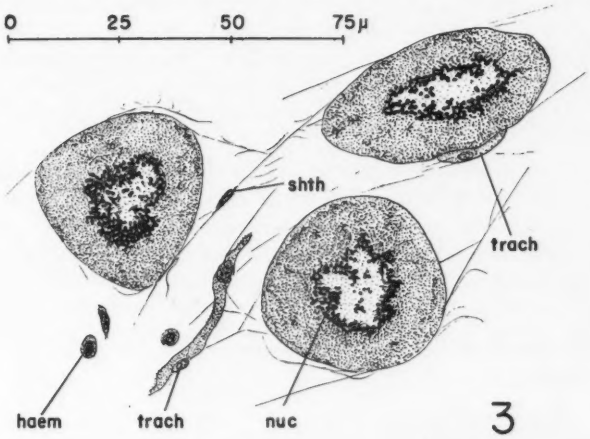



FIG. 2. The development and secretion of a subspiracular gland cell during first instar. A. At eclosion. B. Twenty-four hours after eclosion. C. In a 2- or 3-day-old larva. D. In a 4-day-old larva (approximately 24 hours before molting). E. At the moment of the first larval molt.

the body, i.e. 15 to 25 $\mu$ by 8 to 18 $\mu$. The cytoplasm is loosely granular, containing a meshwork of a thread-like reticulum which appears to radiate from the nucleus. The nucleus is round to oval, 8 to 10 $\mu$ by 8 to 10 $\mu$; the nucleoplasm is coarsely granular. During the next 4 to 5 days (the length of time depending on the individual), these cells follow a definite development pattern leading to a secretion just before the first larval molt (Fig. 2). The cells enlarge rapidly at first, then more slowly, reaching a size of 30 to 50 $\mu$ by 25 to 45 $\mu$ 12–14 hours before the molt (Fig. 2C). The nucleus follows a similar pattern, enlarging to a size of 15 to 25 $\mu$ by 15 to 18 $\mu$. It is round to oval and lacks a nucleolus until about two days before the molt. At that time, the nuclear membrane seems to disappear, while the shape of the nucleus becomes irregular and indistinct (Fig. 2D); the nucleoplasm remains coarsely granular. The increase in size seems mainly due to an increase in the unstained matrix, the coarse, stained particles spreading farther and farther apart. Between 12 and 24 hours before the molt large, clear vacuoles appear around the irregularly shaped nucleus (Fig. 2D). The nucleus now contains a clearly visible nucleolus. As the vacuoles appear in the cytoplasm the nucleus shrinks. At the moment of molting it appears as a very dense, darkly stained body, 7 to 30 $\mu$ by 5 to 8 $\mu$ (Fig. 2E). The vacuoles, which 6–12 hours before molting are filling the cytoplasm, quickly and completely disappear.

The cell shrinks, becoming pear- or even spindle-shaped, but with elongated ends. The cell size is now 25 to 40 $\mu$ by 10 to 25 $\mu$. The cytoplasm is very dense and granular in spots.

In the following instars this general pattern of secretory activity repeats itself. During the second instar the gland cells reach a size of 45 to 125 $\mu$ by 30 to 45 $\mu$, with the nuclei 20 to 50 $\mu$ by 10 to 18 $\mu$ (Fig. 3). At the time of the second larval molt, the nucleus again shrinks into a small, dense body.



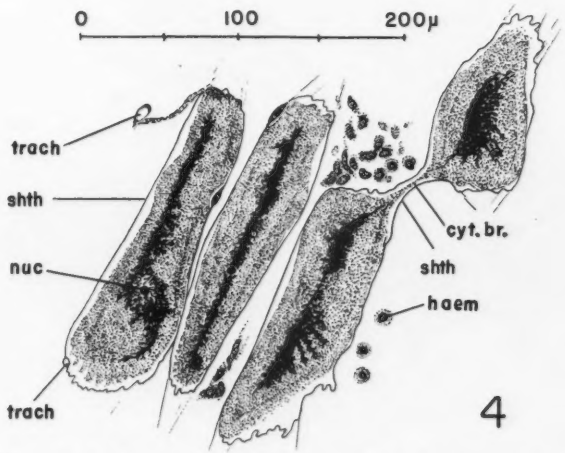




FIG. 3. Three gland cells and surrounding tissues from a subspiracular gland in a late second instar larva.

FIG. 4. Four gland cells and surrounding tissues from a subspiracular gland in a late fifth instar larva.

This time its shape is much more irregular, with many narrow, sharp, often branched projections. Again, a nucleolus is visible, and vacuoles appear in the cytoplasm. The vacuoles disappear shortly after, leaving the cells as smaller, dense, darkly stained, irregularly shaped bodies. In the third instar the events observed in the second instar are repeated.

In the fourth and fifth instars, and in the pupa, events connected with the secretion are basically the same as in the previous instars. The cells, however, are much larger, and though they are of a simple shape, i.e. round, oval, pear- or spindle-shaped, the nuclei are not. Gradually, the nuclei have become more and more irregular in shape, until, in the later larva and pupa, they may be described as disk- or ribbon-like structures with deeply branched and lobed edges (Fig. 4). Each nucleus contains one to three large (up to 8 $\mu$), round nucleoli.

The larva stops feeding approximately eight days before pupation, and 24 to 36 hours after begins to spin a cocoon. The spinning takes 3 days, after which the animal lies in the cocoon without much outward change until pupation. Pupal structures, including the pupal cuticle, begin to develop internally when the animal stops spinning.

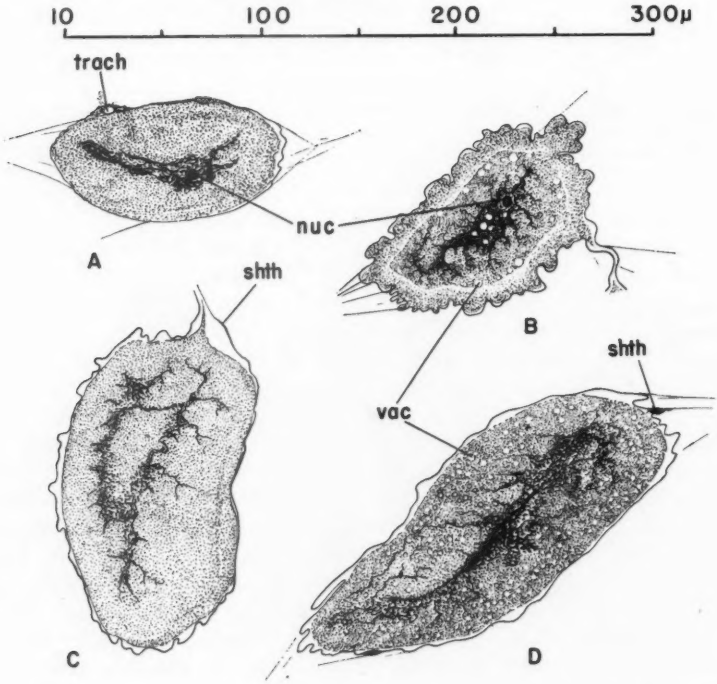



FIG. 5. The development and secretions in a prepupa and in an early pupa. A. Gland cell in a "spinning" larva. B. Secreting gland cell 3 days before pupation. C. Cell 36 hours before pupation. D. Secreting gland cell immediately after pupation.

During the first 2 days of the period from cessation of feeding until pupation, the subspiracular gland cells are found to be similar to those of the late feeding fifth instar larva (Fig. 5A). Nucleoli appear shortly before the cessation of feeding. After spinning has commenced, the cell membrane and the overlying sheath becomes more and more irregular in shape. Three days before pupation the cells are strongly amoeboid in outline (Fig. 5B). At the same time, the nucleus branches extensively; nucleoli are now absent. The cytoplasm shows a marked division into inner and outer granular layers, separated by a non-granular hyaline layer. The nuclear branching and the cytoplasmic layering reach their maximum development 3 days before pupation (Fig. 5B). At this stage vacuoles appear in the nucleus and inner cytoplasm. Two days before pupation the amoeboid shape, the nuclear branches, the cytoplasmic layering, and the vacuoles are still present, but to a much lesser extent. One day before pupation the gland cells return to almost their non-secretory appearance (Fig. 5C).

Animals killed during pupation, or 24 hours before or after, have gland cells with no layered cytoplasm, no nucleolus, no amoeboid shape, but with an extensively branching nucleus, and many small vacuoles in the cytoplasm (Fig. 5D). In addition to the many vacuoles, some small wedge- or rod-shaped

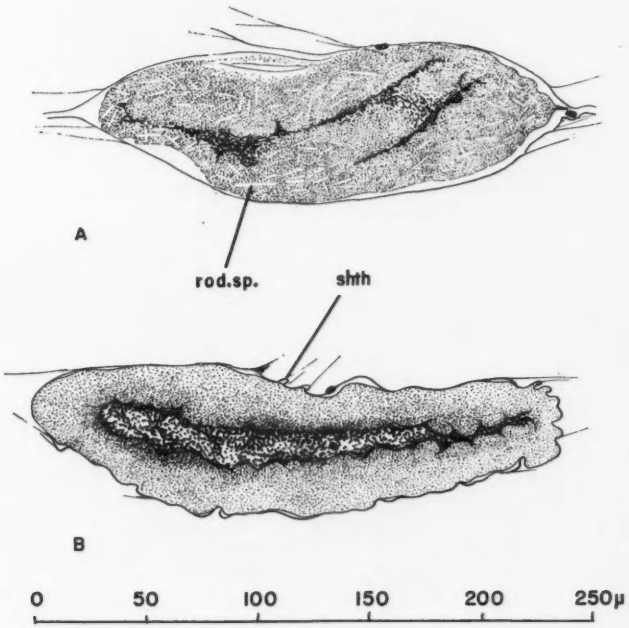



FIG. 6. The subspiracular glands after pupation. A. A gland cell 36 hours after pupation. B. A gland cell 6 days after pupation.

spaces are observed in the cytoplasm. Their appearance suggests that crystals of some sort had been present but were dissolved out in the furan, alcohol, or xylol used to treat the cells before sectioning. At 1 or 2 days after pupation, the number of these spaces is much larger (Fig. 6A), the number of vacuoles is much smaller, the nucleus is less branched, and no nucleoli are present. At 6 days after pupation, and in all later stages, no spaces or vacuoles are found, a nucleolus is now present and the nucleus has the appearance it will keep throughout diapause (Fig. 6B). The gland cells in the pupa have reached a size ranging from 150 to 600 $\mu$ in length and 60 to 150 $\mu$ in width.

Within 24 hours after breaking diapause, the nucleus again branches and vacuoles appear in the cytoplasm; a nucleolus is still present (Fig. 7A). Four days after this activity, the nuclei are less branched and no nucleolus is observed. At approximately 10 days after the breaking of diapause, the glands histolyze (Fig. 7B).

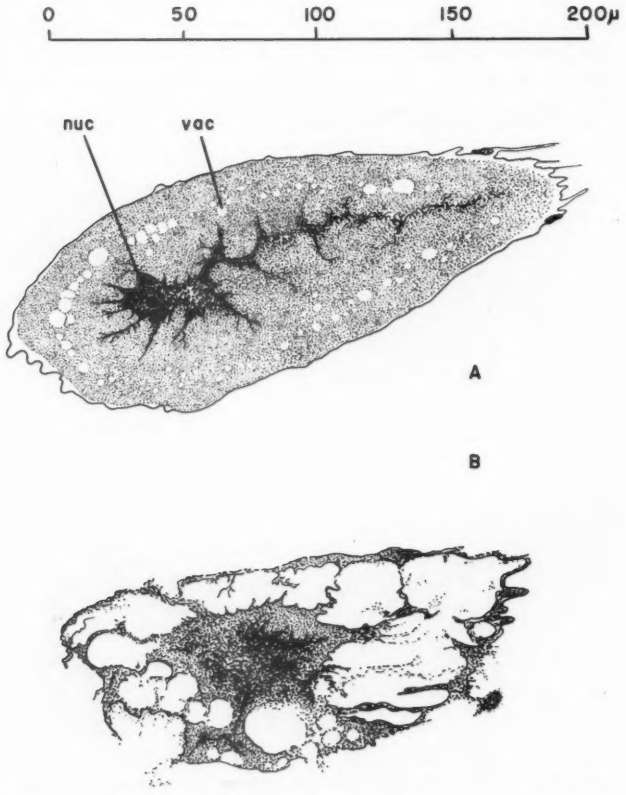



FIG. 7. The subspiracular glands in a pharate imago. A. Secreting gland cell 24 hours after breaking diapause. B. Histolyzing gland cell 9 days after breaking diapause.

*2. The Constriction Division*

Evidence of constriction division is observed shortly after each larval molt. The events are best seen in the later instars. As no live material has been observed in division, the following conclusions are based on a series of fixed cells in various stages of division.

The shape of a resting cell at 24 hours after the molt is spindle-shaped or sometimes oval, but with slender pointed ends. The nucleus appears as a ribbon- or disk-shaped body with branched and lobed edges. Thirty-six hours after the molt various situations are observed which are considered to represent division. Some cells are found with both cell and nucleus constricted (Fig. 9), while others show the typical constricted shape, but with each half containing a complete nucleus (Figs. 4 and 12). In some cases, however, these separate nuclei remain joined with a thin thread of nuclear material. Cells in a later phase are constricted to such a degree that they are virtually two cells connected by a narrow cytoplasmic bridge (Fig. 11), in which a thin strand of nucleoplasm is often observed. When the daughter cells move apart, due to the growth of the larva, the cytoplasmic bridges may attain a length of over 100 $\mu$. In the earlier instars most of the cytoplasmic bridges do not seem to persist. In such situations up to five daughter cells are often found lying side by side. After the growth period in the fifth instar, large numbers of gland cells are found connected by cytoplasmic bridges (Fig. 4). It appears, judging by their number, that most of these

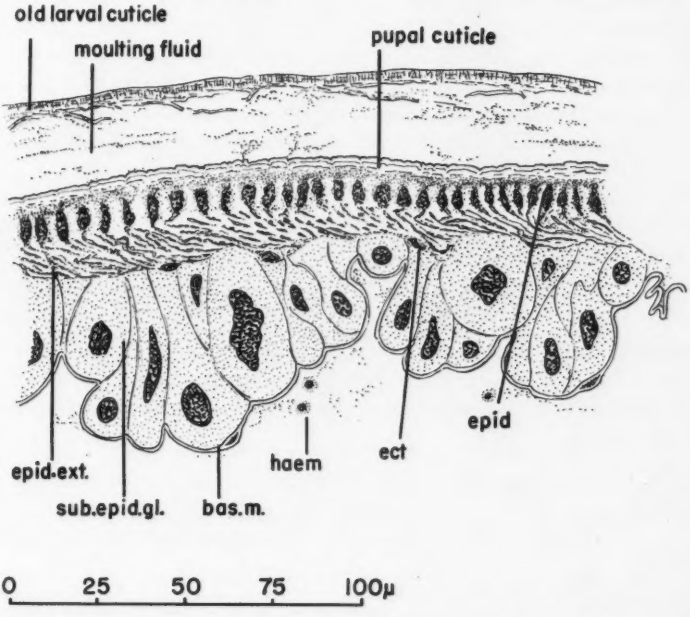



FIG. 8. Cross section through the integument of a prepupa, 29 hours before pupation.

connections are not the result of an incomplete division of this instar, but are connections that already existed in a previous instar which have swollen and thus become visible in the fifth instar. Thus the whole gland is a large syncytium with each unit oval or spindle-shaped and grooved longitudinally.

## The Cytology of the Tracheal and Sheath Tissues

From the moment of emergence until late in the third instar, the sheath tissue is represented by only a fine network of nucleated filaments attaching the gland cells to each other and to surrounding tissue (Fig. 3). The nuclei are very similar in appearance to the nuclei of haemocytes. It is difficult, if not impossible in many cases, to define them as blood cell or sheath nuclei. The tracheal tissue is represented by a finely divided branch of the tracheal system. The tracheoles enter the glands and are attached to the sheath tissue (Fig. 3).

During the growth periods of the third, fourth, and fifth instars, as described in the section on histology, the tracheal and sheath tissues develop extensively. At the end of the growth period of the fifth instar the sheath completely envelops the glandular tissue (Fig. 4). This sheath is very thin, i.e. less than 1 $\mu$ in thickness. In various places numerous sheath nuclei are found, often concentrated near the slender pointed ends of the gland cells. These nuclei are small, dense, and either oblong or oval (Fig. 4). The sheath of the subspiracular gland is continuous with the lining of the ectotrachea.

Small tracheoles and tracheal filaments are found in great profusion between the glandular elements. These final extremities of the tracheal system are still attached to, and penetrate into, the now complete sheath (Fig. 4). The tracheal nuclei can be distinguished from the sheath nuclei; the former are disk-shaped bodies with a sparse, granular nucleoplasm.

During the periods of growth of the sheath and tracheal tissues the number of blood cells in and around the glands is greatly increased. It is possible that these blood cells are directly involved in the synthesis of the sheath and tracheoles. The blood cells are often found lined up in strands, which appear to be much like unfinished sheath or tracheal filaments (Figs. 13 and 14).

## Discussion and Conclusions

This study has made it clear that the subspiracular glands in the larva and pupa of *Hyalophora* are true organs. From the events observed during the development of the insect it appears that the glands secrete cyclicly.

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FIG. 9. Constricted gland cell in a second instar larva. Note that the nucleus is still continuous.

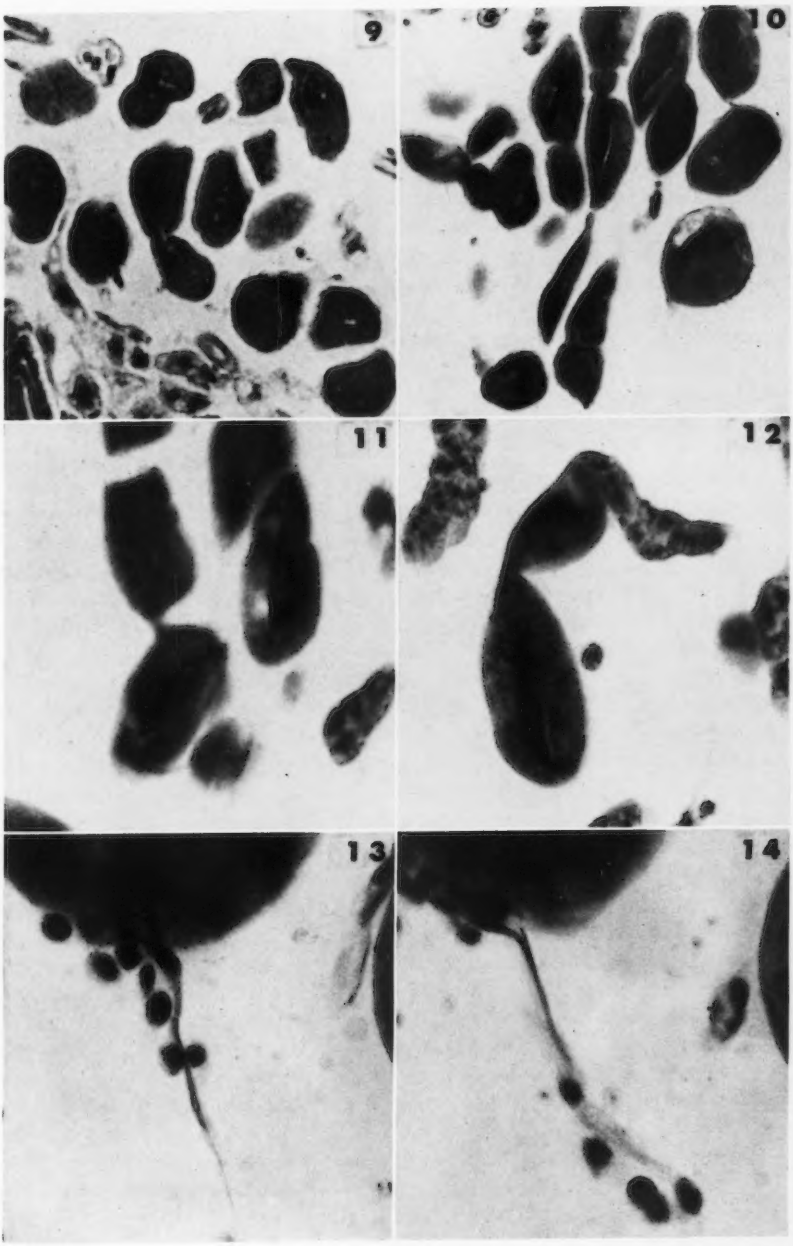
FIG. 10. Strand of gland cells connected by cytoplasmic bridges in a fourth instar larva.

FIG. 11. Two separate gland cells connected by a cytoplasmic bridge in a late fourth instar larva.

FIG. 12. Strongly constricted gland cell in a fourth instar larva.

FIGS. 13 and 14. Two subsequent sections through developing subspiracular gland sheath tissue in a fourth instar larva. Note the conspicuous congregation of haemocytes around the incomplete filaments.

PLATE I




Harmsen and Beckel—Can. J. Zool.

The secretions take place at the following times: in the fetal larva just prior to eclosion (10); in the larva before each molt and before pupation; in the pupa just after pupation; in the pharate imago directly after breaking diapause. All secretions are preceded by an enlargement of the nucleus in the earlier instars and by a profuse branching of the nucleus in the later instars. A nucleolus is observed prior to and during each secretion except for the secretions before and after pupation. Because these latter secretions follow one another very closely one might believe that there are not two separate secretions, but only one, which may occur from 3 days before until 1 day after pupation. However, the presence of rod-shaped spaces which were observed in the pupal, but not in the prepupal secretion is evidence for two separate secretions. As further evidence, there is the layered cytoplasm and the amoeboid-shaped membrane of the gland cells observed during the pupal secretion. Also suggesting two secretions is the observation of an intersecretory period rather than a gradual transition between the prepupal and pupal secretions. That is, in all subspiracular gland cells of specimens killed 1 to 2 days before pupation, no vacuoles were seen in the homogeneous finely granular cytoplasm.

This present investigation of all stages in the life cycle of an insect shows that the subspiracular glands undergo continual change. With this information many of the scattered observations reported in the literature can be correlated. Various authors (5, 11, 15, 26) reported crystalline inclusions in the gland cells, while others reported the absence of such structures (8, 12). In *Hyalophora* inclusions of a probably crystalline nature were encountered only for a brief period shortly after pupation. As the above-mentioned authors worked each with a different insect, and each with usually only one developmental stage of that insect, their inconsistent findings are not surprising. Crystals may appear in various instars depending on the physiology of the insect. In order to discover when (if at all), they appear and to investigate their function, all stages of any one insect must be studied. The enormous increase in size of the gland cells of *Hyalophora* from 6 $\mu$ in length in the embryo to a maximum of 600 $\mu$ in length in the pupa, explains why reports on single stages often disagreed with respect to the size of the gland cells. Also, the considerable and rapid change in the occurrence as well as in the size, shape, and number of such bodies as nucleoli, vacuoles, nuclear branches, cytoplasmic zones, etc. makes it clear why reports on investigations of single specimens often disagree on the cytology of the gland cells.

The term oenocyte has often been applied to at least two types of abdominal gland cells in insects: to the subspiracular gland cells and to the subepidermal gland cells. In Lepidoptera there are subepidermal as well as subspiracular gland cells. Remarks in the literature where the cells are referred to as oenocytes may refer to either type. This leads to contradictory and confusing descriptions if the cells are not precisely located.

In *Hyalophora* a preliminary study of the subepidermal glands was made to define these gland cells in relation to subspiracular gland cells. Subepidermal gland cells arise amitotically from the epidermis in the prepupa. They

lie ventral to, and extend part way up, the body wall in the first five abdominal segments. They are round to oval, or sometimes even pear- or sickle-shaped, and 20 to 35 $\mu$ in diameter when round. They lie between the basement membrane and the epidermis. Their cytoplasm is dense and finely granular, and the nucleus is up to 15 $\mu$ in diameter (Fig. 8).

Shortly after their formation vacuoles are observed in the cytoplasm. After pupation the vacuoles are no longer seen. When the pupa breaks diapause there is again evidence of secretion. During the development of the pharate imago, the gland cells appear to migrate slightly into the fat body. The question of whether they break through the basement membrane, and, if so, the time of break-through has not been established. The subepidermal glands are still found in the adult stage. In all stages they are distinct and different from the subspiracular glands.

With this information and reports from the literature it is possible to form some opinion of the occurrence of subspiracular and subepidermal glands in different orders of insects.

In Diptera both kinds of glands are found. However, after their formation the postlarval subepidermal glands migrate inward to disperse among the fat body cells (6, 12, 14, 16). The situation appears to be similar in Hymenoptera (1, 4, 11, 15, 23, 26, 33). The subspiracular glands in the older Hymenoptera larva are less restricted to segmentally arranged clusters than is the case in Lepidoptera. The postlarval subepidermal gland cells are found to disperse into the fat body in the imago. In Coleoptera the situation varies considerably from one genus to another. Both the subspiracular glands and the subepidermal glands are produced in clusters under the spiracles in *Calandra* (21) and *Dytiscus* (19). In *Tenebrio*, *Harmonia*, and *Melasoma*, however, the subepidermal gland cells are produced by the epidermis throughout the integument (18, 37). In some cases in this order, the subepidermal gland cells migrate inwards into the fat body during imaginal life. In *Sialis* (Neuroptera), typical subspiracular glands are found (22), but no postlarval subepidermal glands are produced.

Wigglesworth (35, 36, 38) has described in detail the development and activity of subepidermal glandular structures in *Rhodnius* (Hemiptera). These cells are first produced in the embryo from the ectoderm under the spiracular invaginations (20). Later a new generation is produced in the epidermis at each molt. The cells are apparently homologous to the subepidermal gland cells of holometabolous insects. A similar situation is reported for *Periplaneta* (Orthoptera) (17).

Thus, it can be concluded that larval subspiracular glands exist, at least for the holometabolous insects of the orders Neuroptera, Lepidoptera, Coleoptera, Hymenoptera, and Diptera. These glands have in common such characteristics as: an early embryonic ectodermal origin ventral and caudal to the spiracular invaginations; a subspiracular location in larva and pupa; cyclic secretions during development; an extremely large size; branching nuclei; repeated constriction division; histolysis in the early imago. It seems

reasonable to assume that these glands are homologous.* The name "subspiracular gland" is suggested as appropriate for these structures to separate them definitely from subepidermal glands.

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*In Hemiptera and Orthoptera similar glandular structures are found in the embryo and fetal larva only. During postembryonic development the glands are replaced by subepidermal structures.

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# CELLULOSE DIGESTION IN THE BEAVER (CASTOR CANADENSIS)[1]

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## Abstract

The digestion of cellulose by the beaver (*Castor canadensis*) has been studied. Indigestible markers added to the feed and artificial caeca were used as techniques in the determination of cellulose digestion. The ability of the experimental animals to digest cellulose ranged from 32 to 33%.

## Introduction

The beaver (*Castor canadensis* Kuhl) is almost the only mammal that includes in its normal diet large quantities of the woody tissue from trees. At the same time, the digestive tract of the animal differs from that of many other rodents in only minor features. The caecum is large but not as large, relative to body size, as that of the guinea pig or rabbit. One of the most characteristic features about the digestive tract of the beaver is the appearance of a large cardiac gland occupying most of the lesser curvature of the stomach (7).

The presence of this peculiar gland, along with the large amount of woody material in the diet, has led to the suggestion that the beaver may produce a cellulase in this organ (19). This possibility was examined in detail by Nasset (15) and by Kitts *et al.* (12), and no evidence of a mammalian cellulolytic enzyme was discovered. Further study in this laboratory (21) has revealed that the beaver may, on the basis of the volatile fatty acid, and sugar levels of the blood, be in the same general category as the ruminants (Table I).

TABLE I

Blood levels of volatile fatty acids and glucose (13, 21)

| Animal | Volatile fatty acid (meq/100 ml) | Blood glucose (mg%) |
|---|---|---|
| Beaver | 0.19 | 49–160 |
| Cow | 0.21 | 40– 60 |
| Dog | 0.03 | 67– 87 |
| Man | 0.03 | 80–100 |

In both of these features the beaver, then, falls into the group of animals in which cellulose breakdown in the digestive tract may be accomplished by the commensal microbiota. It seems reasonably certain that cellulose may contribute to some degree to the energetics of the species.

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Our continuing study of the energy relationships of the beaver, however, necessitated a more detailed understanding of the extent to which the dietary cellulose was utilized for energy. The studies reported upon here were designed to provide this information.

## Methods and Materials

Nine beaver from Vancouver Island were obtained for studies of blood chemistry. Their digestive tracts were made available for the present study. All of the animals were held in captivity for varying periods of time. In addition, the frozen digestive tracts of five beaver dead-trapped in Ontario were available for study.

Animal No. 1 was in captivity 4 months prior to sacrifice. It was refusing food at the time. Animals 2 and 3 were in captivity 3 days and 14 days respectively and were not eating adequately. Animal 4 was in captivity a month, animal 5 only 48 hours; animals 6 to 9 were healthy animals maintained for direct study of feed intake.

It was noted that one constant difference appeared between the captive and the dead-trapped beaver. Without exception, the stomach and upper small intestine of the captive animals were empty while the entire tract of the wild beavers contained food.

Animals in captivity were fed U.B.C. ration 16-57, in pelleted form; the composition of the ration is given in Table II, and the percentage proximate composition in Table III.

TABLE II

Composition of U.B.C. 16-57 ration

| | |
|---|---|
| Ground oats | 400 |
| Ground wheat | 400 |
| Ground barley | 300 |
| Wheat bran | 250 |
| Molasses | 100 |
| Dried grass | 100 |
| Soya meal (45–50%) | 100 |
| Oil cake meal | 100 |
| Copra meal | 100 |
| Fish meal (70%) | 60 |
| Bone meal | 20 |
| Iodized salt | 20 |
| Irradiated yeast | 2 |
| | 1952 lb |

TABLE III

Percentage proximate composition of ration 16-57

| | |
|---|---|
| Moisture | 10.9 |
| Crude protein | 18.1 |
| Crude fiber | 6.3 |
| Cellulose | 5.8 |
| Ether extract | 9.5 |
| N.F.E. (less cellulose) | 43.6 |
| Ash | 5.8 |

Because the rabbit and beaver have somewhat similar digestive tracts, the former animal was included in the present study to evolve the necessary techniques and to afford interspecies comparisons.

The beavers were killed with an overdose of Nembutal injected peritoneally (more than 1.5 ml per lb body weight). Rabbits used were killed by intravenous injection of a saturated solution of magnesium sulphate.

Three types of study were undertaken to determine the level of cellulose digestion undertaken by each animal.

*(a) Artificial Caeca, in vitro Study*

Caecal contents, obtained immediately after the sacrifice of the animal, were diluted to pouring consistency with peptone water and then poured into test tubes 200 mm × 25 mm to a depth of 3 inches. Each test tube contained a piece of rolled cellulose paper 2 inches square. The tubes were first sealed by fitting a rubber balloon over the mouth to collect any gases formed during fermentation, and then were incubated in a water bath at 37° to 39° C for predetermined time intervals.

*(b) Caeca, in vivo Study*

A number of rabbits were subjected to surgery to determine whether parchment paper placed into the living caecum would be digested at the same rate as paper in artificial caeca.

These rabbits were anaesthetized with Nembutal (60 mg/ml) at the rate of 1 cc per 5 lb body weight. Cellulose paper implants were made directly into the caecum via a ventral incision. Each implant was anchored in position by means of thread. The animals were subsequently fed for periods of from 24 to 48 hours before sacrifice. Digestion of the cellulose paper was determined by weight change. One beaver was subject to the same regimen but the results were unsatisfactory and it was decided not to proceed with this aspect of the study until animals more tractable under experimental conditions were available.

*(c) Digestibility Study*

Beavers 8 and 9 were used for a direct determination of the digestibility of their feed. Each of these was kept in a bare room 6 ft × 7 ft with a sloping cement floor. This facilitated the collection of uncontaminated fecal material. Each beaver received feed marked by the addition of 1% of chromic oxide. A 72-hour period was allowed for time of food passage. This exceeded the 60-hour feed passage time determined by Stephenson (20). Beaver 8 was maintained on the marked diet for 2 weeks. It passed no fecal material for 4 days after being moved to the strange surroundings of the experimental room but after that defecated regularly every 24 hours. Beaver 9 was more intractable and fecal passage was irregular.

*Marker Determination*

The method of Schurch *et al.* (18) was used for the determination of chromic oxide in the feed and fecal samples.

Feed and fecal samples were dried in an oven at 100° C or less and then ground in an Arthur H. Thomas Co. grinder using a No. 20 screen. These samples were stored in air-tight bottles. One gram of each of these samples was subjected to analysis. Optical density was calculated for each sample and the milligrams of chromic oxide per gram of feed or feces was read off the standard curve. Feed samples were taken from the feed container prior to feeding and from the feed dish at varying intervals. Fecal samples were collected for 2 weeks for the beaver and rabbits.

*Cellulose Determination*

Cellulose in the feed and feces was determined using the method of Crampton and Maynard (3).

*Apparent Digestibility*

Substitution of the chromic oxide values in the equation of Reid *et al.* (17) allows the calculation of the apparent digestion coefficient for cellulose in the dry material without a knowledge of the total quantity of feces produced or feed consumed.

Apparent digestibility was calculated for cellulose in beaver 8 for 10 consecutive days, in beaver 9 for 14 days, and rabbits 1 and 2 for 14 days.

## Results

*(a) Artificial Caeca*

The amount of apparent digestion given in Table IV is the change in weight of the parchment paper during a specified time interval. The control values show that parchment paper exposed to a salt solution and a warm temperature loses a small percentage of its weight. On the average, this weight loss was equivalent to an apparent cellulose digestion of 3.9%. The present experiments do not permit an explanation of the nature of this weight loss.

In the case of the artificial caeca experiments with the rabbit little or no evidence could be found of cellulose digestion within the first 24 hours of incubation. After prolonged incubation for periods of 48 to 130 hours, there is very definite evidence that cellulose digestion has occurred. In some cases, 25% or more of the available cellulose was apparently degraded. This result confirms the earlier finding by Hall (9). The interpretation of these findings in terms of the whole animal are, however, difficult. The inescapable conclusion is that there is a microbiota present that is capable of digesting cellulose under the conditions provided in the artificial caeca. It is not possible to determine whether corresponding cellulolytic changes occur in the caecum of the animal. This is so because one cannot preclude the possibility that marked microbiotic changes may have occurred using an in vitro technique.

A careful examination of the beaver data reveals that a similar situation exists. In our hands no measurable digestion of cellulose took place during the first 24 hours of in vitro incubation. Prolonged incubation, however, led to definite evidence of cellulolytic activity. Again one cannot be certain that this did not arise as a result of changes in the microbiota arising during

TABLE IV

Some results obtained using artificial caeca to demonstrate cellulose digestion

| Animal | Paper wt. (g) | Time in water bath (hours) | Salvage wt. of paper (g) | Change in paper wt. (mg) | Digestion (%) |
|---|---|---|---|---|---|
| Rabbit 1 | 0.1522 | 24 | 0.1429 | − 9.3 | 6.1 |
| | 0.1545 | 48 | 0.1390 | −15.5 | 10.3 |
| | 0.1572 | 72 | 0.1381 | −19.1 | 12.2 |
| | 0.1526 | 96 | 0.1327 | −19.9 | 13.0 |
| | 0.1552 | 120 | 0.1248 | −30.4 | 19.6 |
| Rabbit 2 | 0.1543 | 24 | 0.1513 | − 3.0 | 1.9 |
| | 0.1565 | 48 | 0.1520 | − 4.5 | 2.9 |
| | 0.1508 | 72 | 0.1358 | −15.0 | 9.9 |
| | 0.1559 | 96 | 0.1427 | −13.2 | 8.5 |
| | 0.1499 | 120 | 0.1370 | −12.9 | 8.6 |
| Rabbit 3 | 0.1543 | 24 | 0.1513 | − 3.0 | 1.9 |
| frozen 24 hours | 0.1565 | 48 | 0.1520 | − 4.5 | 2.9 |
| | 0.1508 | 72 | 0.1358 | −15.0 | 9.9 |
| | 0.1559 | 96 | 0.1427 | −13.2 | 8.5 |
| | 0.1499 | 102 | 0.1370 | −12.9 | 8.9 |
| Rabbit 4 | 0.1569 | 24 | 0.1520 | − 4.9 | 3.1 |
| frozen 48 hours | 0.1496 | 48 | 0.1348 | −14.8 | 9.9 |
| | 0.1483 | 72 | 0.1213 | −27.0 | 18.2 |
| | 0.1557 | 96 | 0.1212 | −34.5 | 22.2 |
| | 0.1567 | 120 | 0.0637 | −93.0 | 59.3 |
| Rabbit 5 | 0.1518 | 24 | 0.1381 | −13.7 | 9.0 |
| frozen 72 hours | 0.1490 | 48 | 0.1342 | −14.8 | 9.9 |
| | 0.1567 | 72 | 0.1391 | −17.6 | 11.2 |
| | 0.1577 | 96 | 0.1337 | −24.0 | 15.2 |
| | 0.1622 | 130 | 0.1373 | −24.9 | 15.4 |
| Beaver control | 0.1486 | 24 | 0.1432 | − 5.4 | 3.6 |
| | 0.1480 | 48 | 0.1444 | − 3.6 | 2.4 |
| | 0.1470 | 72 | 0.1398 | − 7.2 | 4.9 |
| | 0.1511 | 96 | 0.1412 | − 9.9 | 6.6 |
| | 0.1458 | 130 | 0.1432 | − 2.6 | 1.8 |
| Beaver 5 | 0.1554 | 12 | 0.1575 | − 2.1 | — |
| | 0.1570 | 24 | 0.1611 | − 4.1 | — |
| | 0.1524 | 36 | 0.1528 | − 0.4 | — |
| | 0.1523 | 48 | 0.1525 | − 0.2 | — |
| | 0.1542 | 60 | 0.1464 | − 7.8 | 5.1 |
| | 0.1544 | 72 | 0.1400 | −14.4 | 9.3 |
| | 0.1523 | 84 | 0.1283 | −24.0 | 15.8 |
| | 0.1551 | 96 | 0.1230 | −32.1 | 20.7 |
| | 0.1524 | 108 | 0.1047 | −47.7 | 31.3 |
| | 0.1532 | 120 | 0.1160 | −37.2 | 24.3 |
| | 0.1546 | 120 | 0.1385 | −26.1 | 16.9 |
| | 0.1556 | 132 | 0.1217 | −33.9 | 21.8 |
| | 0.1530 | 144 | 0.1155 | −37.5 | 24.5 |
| Beaver 5 | 0.1560 | 5 days | 0.1356 | −20.4 | 13.1 |
| frozen 9 days | 0.1547 | 5 days | 0.1356 | −23.1 | 14.9 |
| | 0.1536 | 5 days | 0.1235 | −30.1 | 19.6 |
| | 0.1546 | 5 days | 0.1385 | −16.1 | 10.4 |
| | 0.1534 | 5 days | 0.1342 | −29.2 | 19.0 |
| | 0.1530 | 5 days | 0.1327 | −20.3 | 13.3 |

incubation. The present data demonstrate that the caecum of a beaver does contain microorganisms that are capable of fermenting cellulose. Stephenson (20) and Currier (4) have shown that the time of food passage of the beaver is in excess of 60 hours. It was for this reason that we incubated the artificial caeca for prolonged periods even though we recognize that the results could

not be unequivocal. Further, the present data show that the cellulolytic microbiota survive freezing and storage.

*(b) Digestibility Study*

The use of indigestible markers as additives to the feed has proved to be a useful technique in determining digestibility. Such substances permit the direct measurement of the degree of concentration of the marker achieved during passage and hence, indirectly, the measurement of the removal of the digestible portion.

Chromic oxide is the substance in common use. Several tests of the efficiency of the chromic oxide technique have been made, and recoveries averaging 95% (5, 14) and 100% (10) have been reported.

The cellulose digestion data derived from two beavers and two rabbits are illustrated in Table V. It will be seen that up to the end of 9 days of administering the marked feed wide fluctuations in the calculated cellulose digestion may occur. Beaver 8 had established its cellulose digestion on the marked diet by day 6 while beaver 9 required 9 days to establish a uniform condition of food passage. This closely approximates the time reported by Kane *et al.* (10) in cattle. If the cellulose digestion is regarded as the mean achieved by the two experimental animals between day 10 and the termination of each trial, beaver 8 demonstrated a mean value of 33% and beaver 9 a mean value of 32%. For comparison, rabbit 1 digested 31% of the cellulose in the marked feed between day 10 and 14. Rabbit 2 did not adjust satisfactorily to this experimental regimen.

The figures in Table V are of the same order of magnitude as those obtained using prolonged incubation of the artificial caeca.

TABLE V

Cellulose digestion by the beaver and rabbit

| Day | Digestion of cellulose (%) | | | |
|---|---|---|---|---|
| | Beaver No. 8 | Beaver No. 9 | Rabbit No. 1 | Rabbit No. 2 |
| 1 | — | 0 | 7 | 0 |
| 2 | — | 0 | 20 | 0 |
| 3 | — | 0 | 14 | 26 |
| 4 | — | 0 | 0 | 13 |
| 5 | 0 | 27 | 27 | 17 |
| 6 | 17 | 8 | 9 | 0 |
| 7 | 29 | 4 | 32 | 0 |
| 8 | 22 | 29 | 44 | 0 |
| 9 | 25 | 0 | 60 | 31 |
| 10 | 43 | 44 | 40 | 34 |
| 11 | 38 | 34 | 27 | 14 |
| 12 | 19 | 25 | 37 | 0 |
| 13 | 37 | 25 | 25 | 0 |
| 14 | 27 | 31 | 25 | 10 |
| Total* | 164 | 159 | 154 | 58 |
| Mean | 33 | 32 | 31 | 12 |

*Calculated from day 10 to termination.

On the basis of the evidence derived from the present work, it would appear that the beaver is able to derive energy from cellulose through the intervention of the microbiota. Present results do not permit the conclusion that the beaver can derive energy from the woody portion as distinct from the vegetative parts.

The fact that cellulose is digested is of interest; however, if the digestion is of no practical use to the animal, such as the production of energy, it is of little value. Stephenson (20) calculated that a 26-lb beaver requires 850 calories of digestible energy obtained from 1.8 to 2 lb of pelleted ration per day, for maintenance. More recently, after using more refined techniques, Pearson (16) has suggested that this value probably should be 760 calories of digestible energy per day. The beaver in this study demonstrated the ability to digest 30% of the available cellulose. Aspen contains 28.07% crude fiber (2), which could if completely digested give rise to 522 calories of available energy per pound of dry aspen. Given a 30% digestiblity, the cellulose or crude fiber in aspen would make available to the animal 156 calories or about 18% of the maintenance energy needs. This is a relatively large amount and it could make a definite contribution to the animal's energy needs in the field. Present findings are compared with others in Table VI.

TABLE VI

Reported cellulose digestion by various species

| Species | Reported digestion of available cellulose (%) | Authority |
|---|---|---|
| Sheep | 60 | Gray (8) |
| Sheep | 82 | Woodman *et al.* (22) |
| Horse | 21–57 | Kellner (11) |
| Rat | 48–55 | Conrad *et al.* (1) |
| Pig | 41–68 | Forbes and Hamilton (6) |
| Pig | 82 | Woodman *et al.* (22) |
| Rabbit | 24–34 | Hall (9) |
| Rabbit | 31 | Present paper |
| Beaver | 32–33 | Present paper |

It remains to demonstrate that the cellulose of aspen can be utilized to the same extent as the cellulose fraction in the pelleted feed offered in the present experiments.

The present study reveals that the beaver, despite its predominantly woody diet, has not developed any special mechanisms for handling cellulose not possessed by a wide range of other species.

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# A VEGETATIVELY PROPAGATED STRAIN OF LARVAL ECHINOCOCCUS MULTILOCULARIS[1]

G. LUBINSKY

## Abstract

Quantitative observations on the growth rate of vegetatively propagated strains of larval *Echinococcus multilocularis sibiricensis* in cotton rats and white mice are reported, and some peculiarities of growth in these animals are discussed. It is shown that the weight of 70-day-old cysts of the seventh transfer in cotton rats was about 25% of the total weight of the host animals, and that the fast growth and the elimination of the hazardous work with definitive hosts make this strain suitable for chemotherapeutic studies.

## Introduction

The use of experimental infections of intermediate hosts for biological, biochemical, and chemotherapeutic studies on echinococcosis (hydatid disease) necessitates the development of techniques assuring the rapid production of a considerable quantity of cysts and the limitation or complete elimination of the hazards involved in working with the definitive host. In a previous paper (1) a description was given of an attempt to establish a vegetatively propagated strain of larval *Echinococcus multilocularis sibiricensis* in cotton rats. To date, this strain has been transferred through 11 vegetative "generations," and substrains in white mice and gerbils have been established. The present paper describes qualitative and quantitative observations made on these strains, special attention being paid to the study of the growth rate of cysts and to the variation of the number of rostellar hooks.

## Materials and Methods

The growth rate of cysts was studied in cotton rats of the seventh transfer. A group of 12 infected animals was sacrificed, 3 on the 30th, 3 on the 50th, and 3 on the 60th day after infection, and the remaining 2 on the 70th day. One rat died naturally on the 36th day. The volume of cysts removed from various locations in the abdominal cavity was measured by immersing them in physiological saline in a measuring cylinder. The growth rate of subcutaneous cysts of the mouse strain was recorded by measuring the size of cysts with a caliper and plotting their outlines on millimeter paper. The variation in the number of rostellar hooks was studied on the basis of the examination of 100 scoleces of each population. Scoleces with anomalies of onchotaxy were rejected in all counts with the exception of that on which the curve in Fig. 14 is based. Figure 11 represents schematically the interrelation of the strains maintained in different hosts.

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## Results

The results of measurements of the volume of cysts in cotton rats of the seventh transfer are represented in Fig. 12. The average volume of cysts on the 30th day after infection was 4.7 cc (variation from 3.1 to 7.9 cc); this volume increased to 15.10 cc on the 50th day and to 19.47 cc on the 60th. The two surviving cotton rats, killed on the 70th day after infection, had an average of 22.3 cc of cysts. The maximum volume, 25.4 cc, was observed in a rat weighing 102.2 g (Fig. 1). The cysts weighed 28.2 g and thus comprised 27.8% of the total weight of the animal. The specific gravity of the cysts was 1.12. However, the specific gravity is not constant and depends both on the density of the population of scoleces and on the amount of calcareous corpuscles present and, therefore, on the age of the cysts. For example, 203-day-old cysts from a gerbil of the second transfer (Fig. 10) were crowded with scoleces and calcareous corpuscles and had a specific gravity of 1.15.

The accumulation of a considerable number of calcareous corpuscles in hydatid cysts makes them comparatively opaque to X-rays and causes them to appear as distinct, mostly circular, shadows on the skiagrams. The enormous number of corpuscles in cysts from the gerbil which died 203 days after intraperitoneal inoculation made the cysts appear as dense shadows on a skiagram taken 5 days before the death of the animal (Fig. 3). Corpuscles in the germinative layer of subcutaneous cysts of a white mouse with a 73-day-old infection made them clearly visible in the X-ray photo, despite the small number of scoleces present (Fig. 4).

Table I shows the distribution of cysts in the abdominal cavity of cotton rats of the seventh transfer. Of practical importance is the fact that from one- to two-thirds of all the cysts are localized on the greater omentum and can be removed easily for explantation or other experiments without the risk of damaging the intestine and contaminating the material.

Numerous cysts were also present on both the gastrohepatic and gastrosplenic ligaments, as well as on the mesenteries. About half of the animals examined had clusters of cysts between the diaphragm and the liver. In almost all of these cases the cysts produced depressions on the surface of the liver. In two cotton rats, of the fifth and eighth transfers, respectively, secondary cysts grew into the liver. In nearly all the animals examined a small cluster of cysts was present on the parietal peritoneum at the point of injection. About 50% of the cotton rats also had subcutaneous clusters at the place of injection. There seemed to exist an inverse correlation between the size of these clusters and the volume of intraperitoneal cysts—the larger the subcutaneous cluster, the smaller the volume of cysts in the peritoneal cavity.

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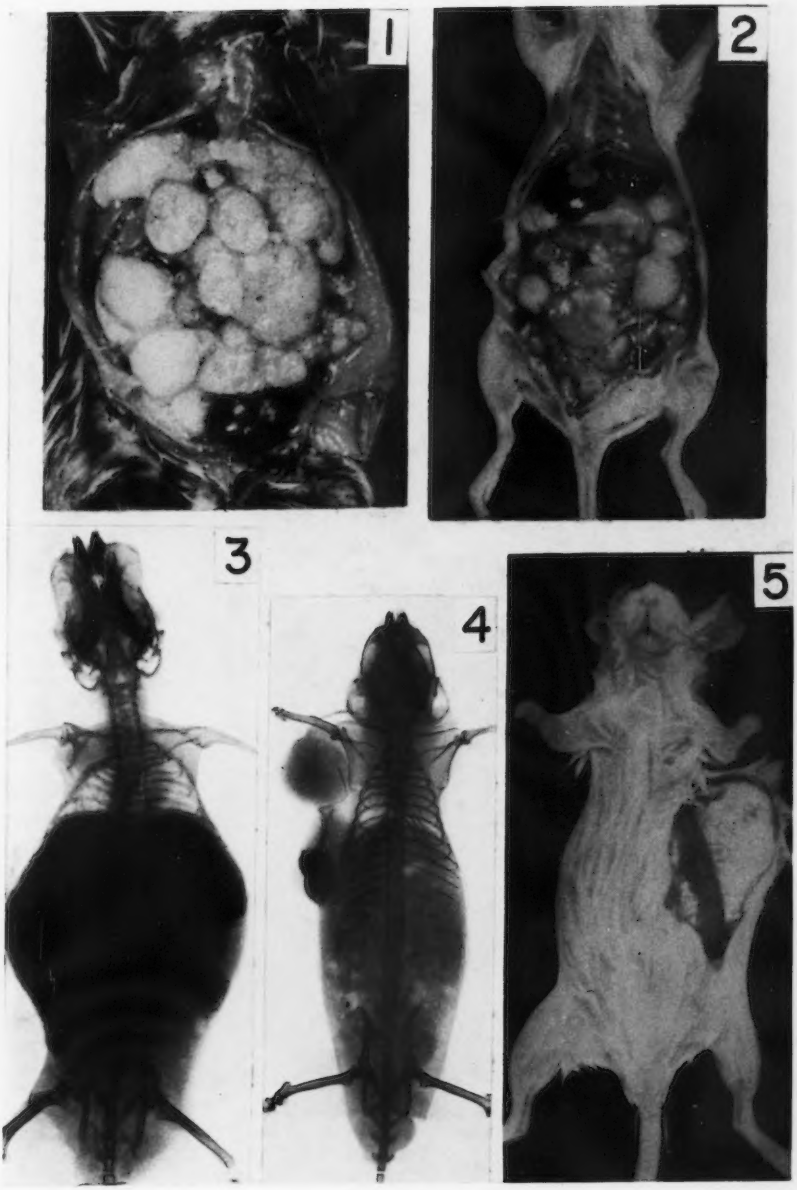
FIG. 1. Intraperitoneal cysts (70 days old) of the ninth transfer in a cotton rat.
FIG. 2. Intraperitoneal cysts (129 days old) of the second transfer in white mice.
FIGS. 3 and 4. Skiagrams of a gerbil with intraperitoneal cysts of the second transfer, 198-day-old infection (Fig. 3), and of a white mouse with subcutaneous cysts of the second transfer (Fig. 4). Dorsoventral direction of rays.
FIG. 5. A 132-day-old subcutaneous cluster of cysts of the second transfer in white mice.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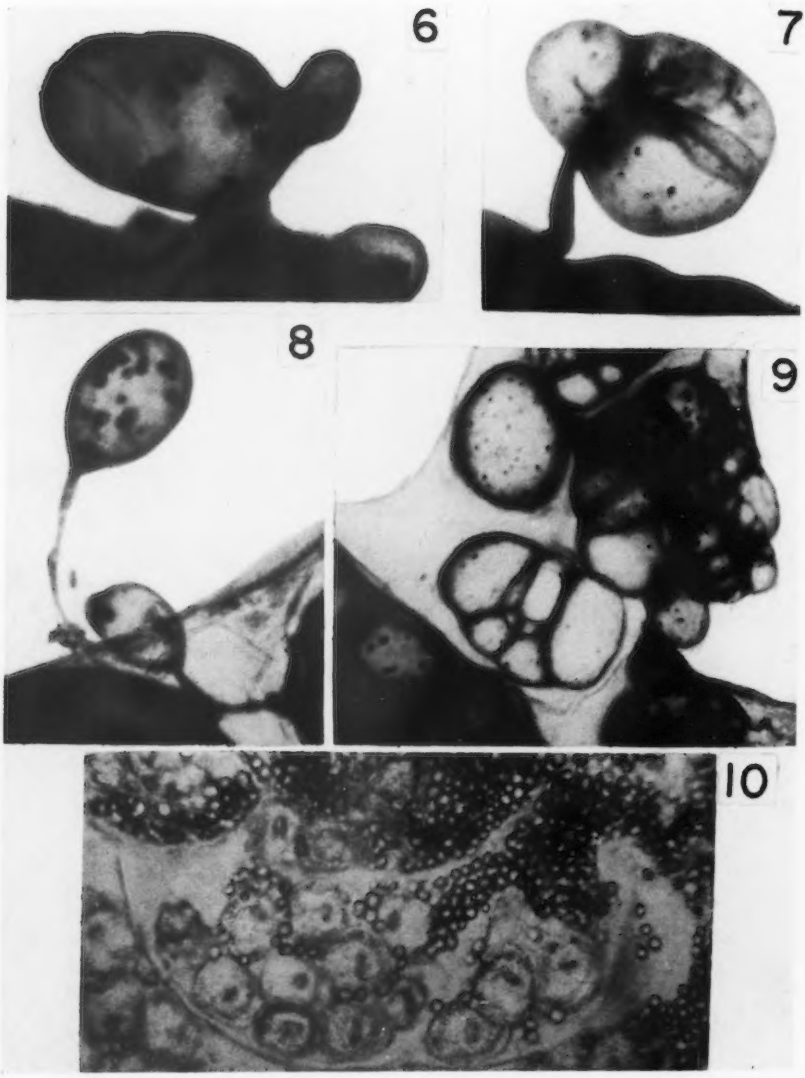


FIG. 6. Cyst of the fifth transfer in cotton rats; length 4 mm.
FIG. 7. Cyst of the seventh transfer in cotton rats; length 3.5 mm.
FIG. 8. Cyst of the sixth transfer in cotton rats; length 0.7 mm.
FIG. 9. Clusters of cysts of the sixth transfer in cotton rats. Length of central cluster 2 mm.
FIG 10. Scoleces in intraperitoneal cysts of the gerbil of the second transfer, a 203-day-old infection.

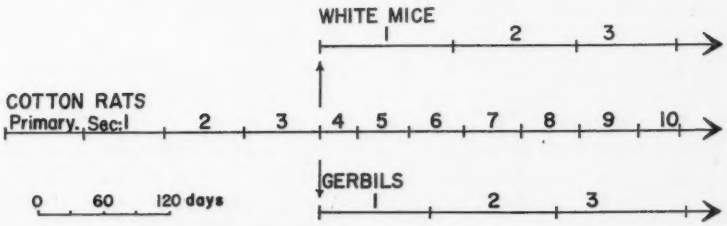


FIG. 11. Interrelations of the strains of larval *Echinococcus multilocularis* maintained in cotton rats, gerbils, and white mice.

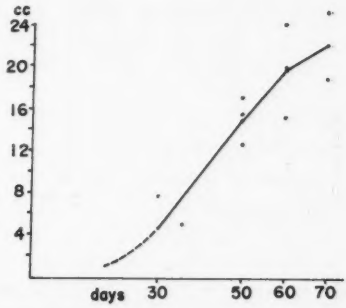


FIG. 12. Growth of the seventh transfer of larval *E. multilocularis* in cotton rats.

The distribution of cysts in the abdominal cavity was, to a certain degree, similar to that of transperitoneal metastases of cancer. The majority of cysts accumulated on the omentum. Cysts on the mesenteries tended to be located near the line of attachment of the mesenteries to the intestine; in animals which often assume an upright position, as for example gerbils and hamsters, cysts tended to accumulate in and near the pelvis. A gentle washing of the abdominal cavity of infected animals always revealed the presence of free scoleces and small free-floating cysts. The relative importance of these two elements in the transperitoneal dissemination of hydatid cysts has not been determined. Yet budding cysts were found frequently in the abdominal cavity (Figs. 6–8), and it seems probable that the production of clusters of cysts similar to those represented in Fig. 9, depends, at least partially, on the process of budding.

In one case a cluster of cysts which developed near the mesenteric attachment of the small intestine attained a size of 16 by 11 by 7 mm and became firmly attached along almost a half-inch of an intestinal loop. Torsion of this cluster resulted in its haemorrhagic infarction, intestinal obstruction, and, finally, in the death of the animal 59 days after infection.

In one cotton rat of the sixth transfer which died 42 days after infection, the entire parietal peritoneum, including that of the pelvis and of the lower surface of the diaphragm, was covered with an almost uninterrupted layer of

TABLE I

Distribution of cysts in the abdominal cavity of cotton rats of the seventh transfer

| Age of infection (days) | Volume of cysts (cc) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | At site of infection | | Greater and gastrosplenic omentum | Mesenteries | Subphrenic space | Gastrohepatic omentum | Other locations | Total |
| | Subcutaneous | On parietal peritoneum | | | | | | |
| 30 | — | 0.2 | 4.7 | 0.3 | — | 2.5 | 0.2 | 7.9 |
| 30 | — | 0.2 | 2.3 | 0.4 | 0.1 | — | 0.3 | 3.3 |
| 30 | 0.3 | 0.1 | 1.3 | 0.1 | — | 1.2 | 0.1 | 3.1 |
| 36 | — | 0.1 | 2.9 | 0.1 | — | 1.3 | 0.7 | 5.1 |
| 50 | 0.1 | — | 8.8 | 1.4 | 0.3 | 2.9 | 0.3 | 13.8+0.2* |
| 50 | — | 0.1 | 9.5 | 4.3 | — | 3.2 | — | 17.1 |
| 50 | 1.7 | — | 6.6 | 3.1 | 2.1 | 2.0 | — | 15.5 |
| 60 | 0.2 | — | 12.1 | 1.9 | — | 5.5 | 0.2 | 19.9 |
| 60 | — | 0.1 | 10.5 | 0.9 | 1.2 | 2.1 | 0.5 | 15.3 |
| 60 | — | 0.2 | 17.6 | 2.9 | 2.1 | 1.4 | — | 24.2 |
| 70 | 0.6 | 0.4 | 18.8 | 2.4 | 2.3 | 0.9 | — | 25.4 |
| 70 | 0.8 | 0.5 | 10.7 | 1.8 | 1.7 | 2.9 | 0.4 | 18.8 |

*On thoracic surface of diaphragm.

minute transparent acephalic cysts. Numerous cysts were present on the mesenteries and on the outer surface of the small and large intestine. The subdiaphragmatic cysts had penetrated into the liver and partially replaced its parenchyma. The omentum and spleen were embedded in a large cluster of cysts 36 by 31 by 8 mm in size. The spleen, of a yellowish color, was atrophic and measured 12 by 2 by 1 mm. It is probable that this atrophy had reduced the resistance of the host to the growing cysts. In this connection it would be of interest to study experimentally the effect of splenectomy on the course of secondary hydatidosis in cotton rats, white mice, and other animals.

Attempts to reproduce experimentally a dissemination of cysts on the parietal peritoneum by adding 10 to 20 units of hyaluronidase to the intraperitoneal inoculum failed, although the injections were well tolerated by the cotton rats.

In two cases the penetration of subphrenic clusters of cysts into the thoracic cavity through the hiatus oesophageus with formation of cysts on the thoracic surface of the diaphragm was observed. In one cotton rat, cysts about 1 mm in diameter were present on the base of the right lung but these were not continuous with clusters in the abdominal cavity.

The subcutaneous inoculation of a suspension of ground cysts results in the development of clusters at the site of injection. Such cysts grow much more slowly than the intraperitoneal ones, attaining at most a volume of 8 to 9 cc after 2 months of growth. These cysts are mostly fertile and have a tendency to grow along the track of the needle and to be elongated or even sausage-shaped, especially when a long needle is used for injections. The cotton rats inoculated subcutaneously may survive for 5 or 6 months, much longer than those inoculated intraperitoneally.

The growth rate of intraperitoneally inoculated cysts in gerbils is lower than that of similarly inoculated cysts in cotton rats, and the volume only one-third to one-half that of cotton rat cysts of the same age. The survival time of secondarily infected gerbils is much longer than that of cotton rats. One gerbil of the second transfer, which died 203 days after infection, weighed 113 g, and had cysts weighing 35 g—31.2% of its total body weight. These cysts were packed tightly with scoleces and contained numerous calcareous bodies (Fig. 10). A roentgenogram of this animal 198 days after infection is shown in Fig. 3. The gerbils are thus the animals of choice for the maintenance of the strain of secondary hydatid with a minimum number of transfers.

In white mice the growth rate of cysts is still lower than in gerbils. The animals inoculated both intraperitoneally and subcutaneously survive as a rule for more than 6 months. Both the intraperitoneal and the subcutaneous cysts may sometimes be acephalic, even after several months of growth. However, they are mostly fertile and often produce a considerable number of scoleces. Figure 13 represents the outlines of subcutaneous cysts in four mice of the second transfer in which the cysts grew fastest. It is obvious that the volume of cysts is not directly proportional to the surfaces outlined, but depends on the shape of the cysts. Thus, for example, mouse No. 4 (lower row) had a much smaller volume of cysts than the ovoidal and subspherical clusters

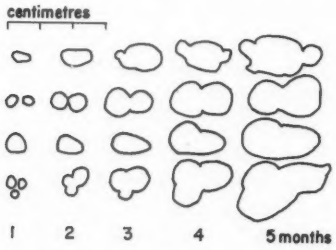


FIG. 13. Outlines of subcutaneous cysts of *E. multilocularis* of the second transfer in white mice taken at 1-month intervals.

of mice Nos. 2 and 3. Figures 2 and 5 represent white mice with intraperitoneal and subcutaneous cysts of the third transfer.

The percentage of "takes" in the third transfer of the subcutaneous cysts in white mice was about 70, as compared with 25 and 40 in the first two transfers. If this percentage and the growth rate of cysts continues to increase in subsequent transfers, the mouse strain may become suitable for chemotherapeutic experimentation.

Several attempts to transfer the cotton rat strain of *E. multilocularis* cysts to guinea pigs and rabbits by both subcutaneous and intraperitoneal injections have failed.

Cysts of all transfers in cotton rats, gerbils, and white mice produced scoleces. A dog was infected successfully with the cysts of the fourth transfer in cotton rats. No attempts were made to infect definitive hosts with cysts of subsequent transfers. The material at our disposal provided an opportunity to investigate the influence of host factors on the variability of the number of rostellar hooks. In the first place it was important to investigate the influence of a series of vegetative transfers on this number. Fifty-eight- to 68-day-old cysts of the second to eighth transfers were ground through wire mesh, the pulp fixed with formalin, and cleared with lactic acid. The counts summarized in Table II are based on scoleces with normal onchotaxy. The mean number of hooks in the populations examined varied from 25.29 to 27.17, thus within narrow limits. However, the difference between the mean numbers in the second and eighth transfers exceeded its probable error four times and was

TABLE II

Number of hooks in the strain of larval *Echinococcus multilocularis* maintained in cotton rats ($n$ = 100)

| Transfer No. | Age of infection (days) | $M \pm \sigma_M$ | $\sigma$, $\pm$ | C.V. (%), $\pm$ | Observed limits of variation |
|---|---|---|---|---|---|
| 2 | 68 | 25.29±0.35 | 3.50 | 13.80 | 14–32 |
| 4 | 61 | 26.12±0.32 | 3.20 | 12.35 | 20–32 |
| 6 | 61 | 27.02±0.21 | 2.06 | 7.62 | 22–32 |
| 7 | 60 | 25.96±0.31 | 3.12 | 13.20 | 16–32 |
| 8 | 58 | 27.17±0.31 | 3.07 | 11.29 | 22–36 |

thus statistically significant. The material at hand is, however, not sufficient to decide whether this difference expresses a tendency of the number of hooks to increase with a progressing number of vegetative transfers.

In conjunction with this phenomenon it was interesting to examine the number of hooks in scoleces from cysts of different age. The strain of secondary hydatid in cotton rats was not suitable for this purpose because the infected animals died within the second month after implantation of cysts. However, the survival time of cotton rats infected with oncospheres varied greatly. I had available material from two cotton rats of the same experiment, one of which died 49 days, the other 134 days after being infected. The average number of hooks in the scoleces from the first rat was 25.90, that from the second, 29.26. The difference of means, 3.36 hooks, exceeded its probable error 8.6 times and thus was highly significant (Table III).

TABLE III

Number of hooks in infections of different age in the strain of larval *Echinococcus multilocularis* maintained in gerbils, and that in cotton rats infected with oncospheres

| Host | Kind of infection | Transfer No. | Age of infection (days) | $M \pm \sigma_M$ | $\sigma$, $\pm$ | C.V. (%), $\pm$ | Observed limits of variation |
|---|---|---|---|---|---|---|---|
| Gerbil | Secondary | 2 | 111 | $25.38 \pm 0.35$ | 3.49 | 13.75 | 18–34 |
| Gerbil | Secondary | 3 | 121 | $25.58 \pm 0.41$ | 4.11 | 16.12 | 16–34 |
| Gerbil | Secondary | 2 | 200 | $29.76 \pm 0.28$ | 2.80 | 9.48 | 20–36 |
| Gerbil | Secondary | 2 | 203 | $29.03 \pm 0.33$ | 3.31 | 11.38 | 18–36 |
| Cotton rat | Oncospheres | — | 49 | $25.90 \pm 0.31$ | 3.12 | 12.35 | 16–34 |
| Cotton rat | Oncospheres | — | 134 | $29.26 \pm 0.23$ | 2.34 | 8.0 | 24–36 |

Adequate material for the comparison of cysts of different age furnished the strain of secondary hydatid maintained in gerbils. These animals survive up to 200 days after intraperitoneal inoculation of this strain. Table III summarizes data concerning the number of hooks in cysts of different age in gerbils of the second and third transfers. Whereas 111- and 121-day-old cysts had scoleces with an average of 25.38 and 25.58 hooks, 200- and 203-day-old cysts had scoleces with an average of 29.76 and 29.03 hooks, respectively. Differences of means between the older and younger cysts exceeded their probable errors 6.5 to 9.6 times and were thus highly significant.

It is obvious that the increase in the average number of rostellar hooks in a population does not depend on the gradual increase in the number of hooks in individual scoleces. Primordia of all the hooks of a scolex appear simultaneously, and no hooks are added in its later development. It can be conjectured that older cysts produce larger scoleces with a larger number of hooks—a conjecture which is obviously in need of confirmation.

It is interesting to note that scoleces from white mice have a higher number of hooks than those from cotton rats and gerbils. For example, the scoleces from a 129-day-old subcutaneous cyst of a white mouse of the third transfer had $31.98 \pm 0.31$ hooks ($\sigma = \pm 3.14$, C.V. $= \pm 9.81\%$), figures close to those for larval scoleces of *E. granulosus* from Alberta moose, and higher than those

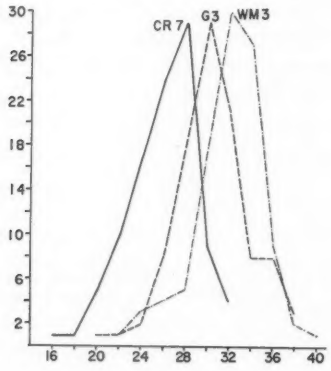


FIG. 14. Variation of the number of rostellar hooks in cysts from the strains of *E. multilocularis*: CR 7, cotton rat strain, seventh transfer, 60-day-old intraperitoneal cysts; G3, gerbil strain, third transfer, 121-day-old intraperitoneal cysts; WM3, white mouse, third transfer, 129-day-old subcutaneous cysts.

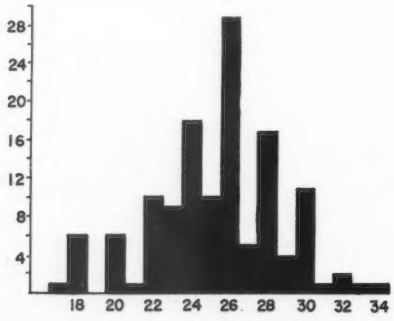


FIG. 15. Variation of the number of rostellar hooks in 130 unselected scoleces from 111-day-old intraperitoneal cysts of the third transfer in gerbils.

for a N.W.T. reindeer (see ref. 2, Table II, p. 607). Figure 14 represents variation curves of the number of hooks in scoleces from this white mouse, from cysts of a gerbil of the third transfer (121-day-old infection), and from a cotton rat with 60-day-old cysts of the seventh transfer. It is noteworthy that the average number of hooks in the scoleces of 129-day-old cysts of the mouse is higher than that from 121-day-old cysts of a gerbil. It thus seems that the number of hooks depends not only on the age of the cysts but also on the species of the host, a fact worth further examination.

Curves of Fig. 14 are based, as already stated, on the examination of scoleces with normal onchotaxy. It may be of some interest to compare these curves with the curve of an 111-day-old infection of a gerbil of the third transfer based on the examination of 130 scoleces taken at random (Fig. 15). In this population the number of crowns with even numbers of hooks by far exceeded that with odd numbers.

The strains of cysts of *E. multilocularis* are further maintained in this Institute in cotton rats, gerbils, and white mice. The growth rate of cysts in all these strains gradually increases, that of cysts of the 10th transfer in cotton rats being considerably higher than the rate in the seventh transfer. It seems probable that in its present condition the vegetatively propagated strain of *E. multilocularis* in cotton rats would permit an evaluation of the activity of chemotherapeutic agents within 1 month, and, at that, without the necessity to work with the oncospheres of this highly pathogenic species.

## Acknowledgment

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# MACROLAIMUS CANADENSIS N. SP. (NEMATODA: PANAGROLAIMINAE), FROM THE FRASS OF THE BARK BEETLE PHLOEOSINUS CANADENSIS SWAINE, 1917, WITH REMARKS ON OTHER SPECIES OF THE GENUS MACROLAIMUS MAUPAS, 1900[1]

K. C. SANWAL

## Abstract

*Macrolaimus canadensis* n. sp., collected from the frass of *Phloeosinus canadensis* Swaine, 1917, infesting a log of eastern white cedar from Lac Tremblant, Quebec, is described and figured. The new species differs from other members of the genus in having a small, thornlike ventral spine on the spicules and in having comparatively stouter cheilorhabdions.

*M. aculeatus* (Daday, 1905) Thorne, 1937, and *M. citri* Rahm, 1928, are regarded as *species inquirendae*. Ruehm's synonymy of *M. hamatus* Thorne, 1937, and *M. crucis* of Fuchs, 1938, with *M. crucis* Maupas, 1900, is not accepted. The systematic positions of *M. crucis* of Ruehm, 1956, and *M. crucis* of Fuchs, 1938, are briefly discussed. *M. maupasi* Hnatewytsch, 1929, is transferred from *Macrolaimus* Maupas, 1900, to *Myolaimus* Cobb, 1920.

Nine male and 10 female nematodes were collected from the frass of *Phloeosinus canadensis* Swaine, 1917, infesting a log of eastern white cedar near Lac Tremblant, Quebec. After careful removal of the bark, the frass was scraped from the galleries and the worms were extracted with a Baermann funnel. Closer study of the worms showed that they represented a new species of the genus *Macrolaimus* Maupas, 1900 (6).

## *Macrolaimus canadensis* n. sp.

*Female*

Length 0.99–1.2 mm, $a = 34–36.6$, $b = 4.5–4.9$, $c = 13.5–14.8$, $V = 55–57.6\%$. Body small and translucent, attenuated only very slightly towards anterior end, more so behind anus. Body narrowing abruptly behind vulva. Cuticle with fine transverse striations, 0.0018 mm apart at middle of body; two lateral incisures.

Head not marked off from rest of body; lips arched over mouth cavity. Six bristlelike papillae on head (Fig. 1).

Mouth opening terminal; buccal cavity spacious, distinctly divisible into a wide anterior chamber (cheilostom) and a narrower posterior chamber (prostom). Behind prostom a shallow, funnel-shaped region (meso-, meta-, and telo-stom) opening into lumen of esophagus. Walls of anteriormost chamber (cheilorhabdions) broad at base, curved in the shape of an arc, very lightly sclerotized, and extending to mouth opening. Walls of posterior chamber (prorhabdions) heavily sclerotized and prominent. Walls of shallow, funnel-shaped region (meso-, meta-, and telo-rhabdions) very thin but well sclerotized.

Esophagus long, enveloping bases of prorhabdions. Precorpus and corpus blended, almost cylindrical; no median esophageal bulb, only a slight swelling

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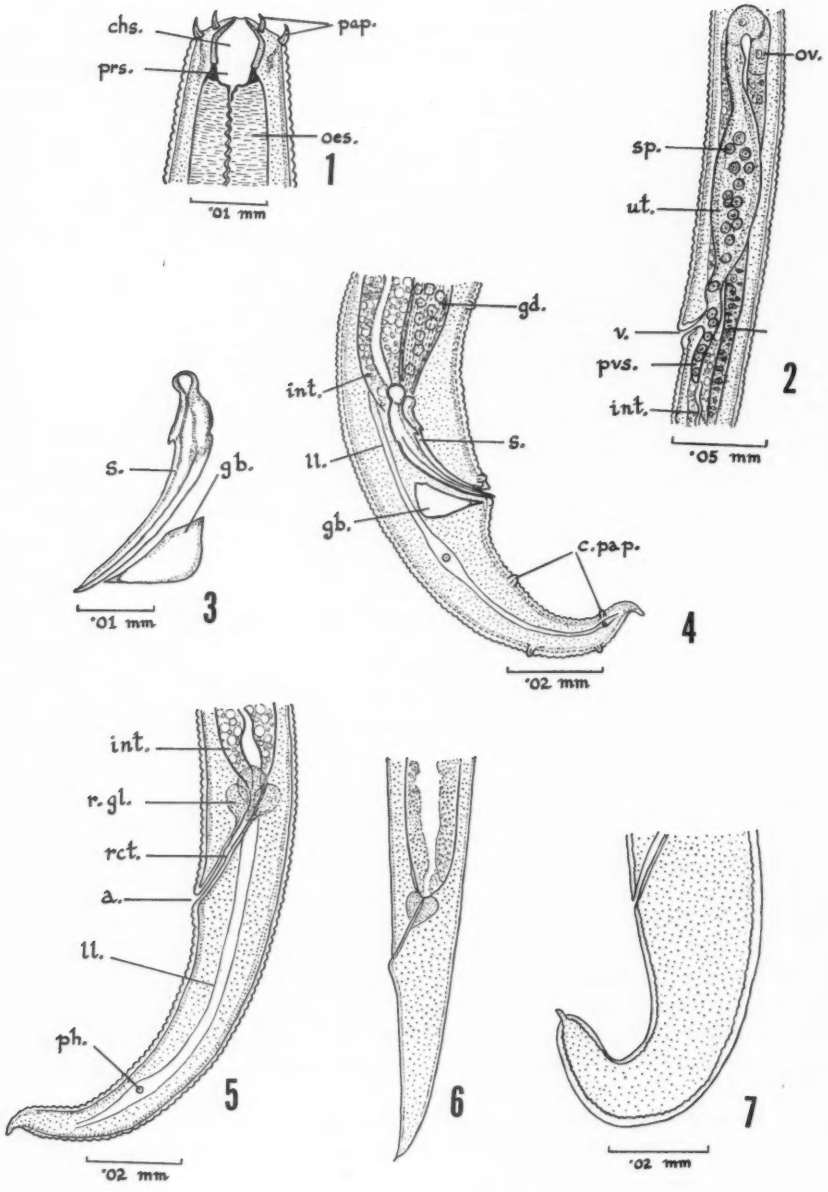
Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.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in its place. Isthmus narrow at first but gradually widening into a valvulated, club-shaped terminal bulb that is longer than broad. Valves showing comb-like structure. Lumen of esophagus sinuous and dilated slightly immediately behind the valve. Well-developed cardia between esophagus and intestine. Lumen of intestine saccate or with caecaelike ramifications at beginning; remainder of lumen a wide, sinuous tube with occasional irregular, longish dilations. Rectum with cuticularized lining and almost twice as long as anal body width. Rectal glands present.

Excretory pore from one to two body diameters posterior to slightly swollen region of corpus. Amphids and nerve ring not observed.

Vulva postequatorial, with prominent anterior lip (Fig. 2). Body diameter diminishing sharply posterior to vulva. Reproductive organs prodelphic, reflexed. Ovary extending back almost to end of intestine. Uterus small, a distended region serving as seminal receptacle and full of sperms in specimens examined. A short postvulval uterine sac present, about one-half to one body diameter long.

Tail almost four anal body diameters long, not narrowing sharply till the extreme end, where it curves dorsally to a bluntly conical point (Fig. 5). Phasmids about one-third of tail length from tip.

*Male*

Length 0.95–1.07 mm, $a=38.8–43$, $b=4.3–4.7$, $c=16.7–19.8$. Cuticle finely striated; striations about 0.0018 mm apart at middle of body; two lateral incisures. Head not marked off from rest of body and bearing six bristlelike papillae. Stomatorhabdion arrangement, esophagus, and cardia as in female. Intestine running parallel to vas deferens. Rectal glands present in region of cloaca. Excretory pore opening one to two body diameters posterior to swollen portion of corpus. Nerve ring surrounding isthmus at about one body diameter posterior to swollen portion of corpus.

Single testis extending anteriorly to about middle of body, where it is reflexed for a short distance. Two spicules present, arcuate, pointed at tips, and each with a small, thornlike ventral spine (Figs. 3, 4). Gubernaculum large, somewhat pyramid-shaped from lateral aspect.

Tail about two and a half anal diameters long, not narrowing sharply till the extreme end, where it curves dorsally to a bluntly conical point (Fig. 4). Position and number of caudal papillae as follows: two pairs preanal, subventral; four pairs postanal, two pairs subventral, and two pairs subdorsal. In addition there are probably two more pairs of papillae, postanal, lateral in position, which are very small and indistinct.

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FIGS. 1–5. *Macrolaimus canadensis* n. sp. 1. Anterior region of male. 2. Vulvar region of female. 3. Spicule and gubernaculum. 4. Lateral view of male tail. 5. Lateral view of female tail.

FIG. 6. *Macrolaimus crucis* Maupas, 1900. Lateral view of female tail.

FIG. 7. *Macrolaimus crucis* of Ruehm, 1956. Lateral view of female tail.

ABBREVIATIONS: *a.*, anus; *c. pap.*, caudal papillae; *chs.*, chilostom; *gb.*, gubernaculum; *gd.*, gonoduct; *int.*, intestine; *ll.*, lateral line; *oes.*, esophagus; *ov.*, ovary; *pap.*, cephalic papillae; *ph.*, phasmid; *prs.*, prostom; *pvs.*, postvulvar uterine sac; *r. gl.*, rectal glands; *rct.*, rectum; *s.*, spicule; *sp.*, spermatozoa; *ut.*, uterus; *v.*, vulva.

*Diagnosis*

*Macrolaimus* with dimensions and general features as described above. Easily distinguished from other species of the genus by the presence of a small, thornlike ventral spine on spicules and comparatively stouter cheilorhabdions.

*Host.*—Associated with the bark beetle *Phloeosinus canadensis* Swaine, 1917, infesting eastern white cedar; collected from frass in galleries.

*Locality.*—Lac Tremblant, Quebec.

Holotype (male) and paratypes in the Canadian National Collection of Nematodes, Nematology Section, Entomology Research Institute, Ottawa, Ontario, collection No. 1623.

## Discussion

*M. canadensis* differs from *M. crucis* Maupas, 1900 (6) in shape and size (more than four times the anal body width in *M. canadensis*) of the female tail (compare Figs. 5, 6), in presence of a postvulvar uterine sac, in having stouter cheilorhabdions, and in the presence of males (Maupas (6) gave definite evidence in support of the absence of males and found that *M. crucis* reproduces parthenogenetically). It differs from *M. hamatus* Thorne, 1937 (11) in size and proportions of body (except vulva percentage), and in having a wider buccal capsule, stouter cheilorhabdions, a postvulvar uterine sac, thornlike spine on each spicule, and in shape and size of gubernaculum. From *M. taurus* Thorne, 1937 (11), the new species differs in all characters enumerated above in connection with *M. hamatus* and also in the extent of cheilorhabdions (these do not reach edge of the mouth in *M. taurus*), and position of vulva (more posterior in *M. taurus*).

*Remarks on Other Species*

*M. aculeatus* (Daday, 1905) Thorne, 1937 (2, 11), and *M. citri* Rahm, 1928 (7), are regarded here as *species inquirendae* for the following reasons: *M. aculeatus*, which is described from one male specimen only, has a peculiar mouth cone and buccal armature. It is doubtful whether this species belongs to the genus *Macrolaimus*. From the description of *M. citri*, without any illustration and based on one female specimen only, it is difficult to determine the correct systematic position of this species. Rahm (8) placed this species in the genus *Macrolaimus* only provisionally.

*M. maupasi* Hnatewytsch, 1929 (5), is transferred to the genus *Myolaimus* Cobb, 1920 (1), on the basis of the original description and illustrations given by Hnatewytsch. The presence of a large tooth in the stoma, well-developed caudal alae in the male tail supported by riblike papillae, a large amphidial aperture, and absence of spicules and gubernaculum exclude this species from the genus *Macrolaimus*. *M. maupasi* Hnatewytsch, 1929, thus becomes *Myolaimus maupasi* (Hnatewytsch, 1929) n. comb.

*M. papillatus* (Rahm, 1928) Goodey, 1951, was transferred to the genus *Chambersiella* Cobb, 1920, by the author in a previous communication (10).

The author does not agree with Ruehm (9) in synonymizing *M. hamatus* and the male worm described by Fuchs (3) as *M. crucis* with *M. crucis* Maupas. It appears that the species described by Ruehm (9) as *M. crucis* is not *M. crucis* as described by Maupas (6), for two reasons: (*a*) Maupas (6) was completely convinced of the absence of males in *M. crucis* as a result of rearing experiments but Ruehm found males in his material; (*b*) the shape of the female tail in *M. crucis* of Maupas is very different from that in Ruehm's species (Figs. 6, 7). Furthermore, the spicules and gubernaculum of *M. hamatus* are different from those in Ruehm's species. Ruehm's specimens, therefore, are not the same as *M. crucis* of Maupas or synonymous with *M. hamatus*.

Regarding the male worm described by Fuchs (3) as the male of *M. crucis* Maupas and thought to be the male of *M. hamatus* by Goodey (4), no definite conclusions can be reached. The male tail figured by Fuchs (3) resembles that of *hamatus* to some extent, but the body narrows suddenly behind the anus in Fuchs' specimens whereas it is gradually attenuated in *hamatus*. Further, Fuchs' species does not conform with Ruehm's species, differing in the shape of the male tail, shape of the spicules, and the length of the female tail. In length of the female tail Fuchs' species corresponds to that of *M. canadensis*.

## Acknowledgments

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# THE CORAL REEFS AND CORAL COMMUNITIES OF BARBADOS, W.I.[1]

JOHN B. LEWIS

## Abstract

Actively growing, fringing coral reefs are developed along the western coast of Barbados. The form of these reefs, from the shore to depths of about 30 meters, is described. *Montastrea annularis* is the dominant shallow water species while *Madracis asperula* is dominant beyond depths of 10 meters. The development of the reefs in terms of coral growth is described.

## Introduction

Barbados lies some 90 miles to the east of the Lesser Antilles. It has a length of 21 miles and is 11 miles wide. The total area is 166 sq. miles and the highest elevation is 1104 feet. It is covered for its largest part by Pleistocene reef limestone (8). In the northern portion of the island this coral cap has been removed by erosion and exposed the older underlying layers of sedimentary rocks (3, 9, 11, 12). The limestone formation rises as a series of terraces, slopes, and scarps. The coastline is regular and there are no deep indentations nor any offshore islands. There are long stretches of calcareous sand beaches around most of the island except at the northern and southern ends where limestone cliffs rise directly from the sea.

The actively growing coral reefs of the island are restricted to the western or leeward coast, between Bridgetown on the south and Shermans, 10 miles to the north. The distribution of the reefs is shown in Fig. 1. They are of the fringing type and form more or less crescent-shaped bands fronting each promontory along the coast (Fig. 2). Shallow bays with a sand or rubble bottom lie between each of the promontories and separate the individual reefs.

No actively growing reefs were seen along the other coasts. Off the southern coast there are extensive rubble banks of calcareous rock but these support at present only widely scattered coral patches. The reefs along the eastern and northern coasts are composed of dead coral rock which supports only secondary growths of coral.

Thirty-three scleractinian species listed below have been collected by the author on reefs in Barbados:

ASTROCOENIIDAE
*Stephanocoenia michelinii* Milne Edwards & Haime

ACROPORIDAE
*Acropora cervicornis* (Lamarck)
*Acropora palmata* (Lamarck)

IOCILLOPORIDAE
*Madracis asperula* Milne Edwards & Haime
*Madracis decactis* (Lyman)

[1]Manuscript received July 9, 1960.
Contribution from the Bellairs Research Institute of McGill University, St. James, Barbados, W.I.

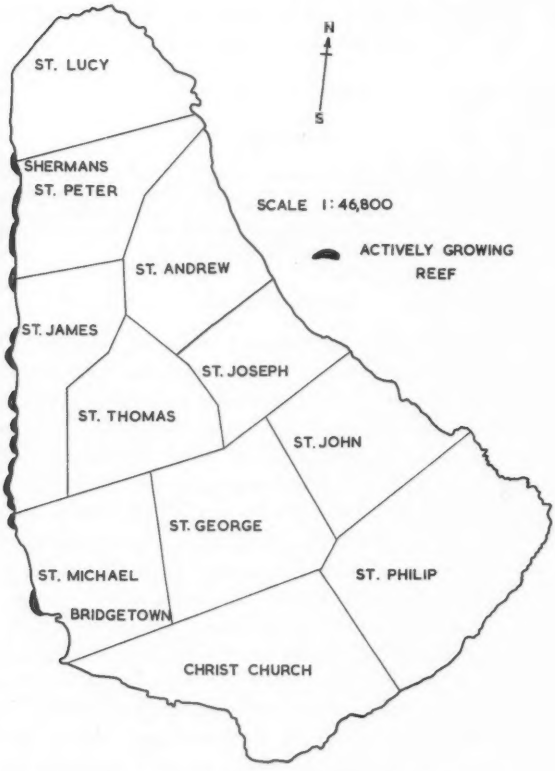


FIG. 1. Map of Barbados showing distribution of reefs.

AGARICIIDAE
*Agaricia agaricites* (Pallas)
*Agaricia fragilis* Dana
*Agaricia nobilus* Verrill

SIDERASTREIDAE
*Siderastrea radians* (Pallas)
*Siderastrea siderea* (Ellis & Solander)

PORITIDAE
*Porites astreoides* Lamarck
*Porites divaricata* Lesueur
*Porites furcata* Lamarck
*Porites porites* (Pallas)

FAVIIDAE
*Favia fragum* (Esper)
*Diploria clivosa* (Ellis & Solander)
*Diploria strigosa* (Dana)
*Diploria labyrinthiformis* (Linnaeus)
*Colpophyllia amaranthus* (Muller)
*Colpophyllia natans* (Muller)
*Manicina areolata* (Linnaeus)
*Montastrea annularis* (Ellis & Solander)
*Montastrea cavernosa* (Linnaeus)

RHIZANGIIDAE
*Astrangia solitaria* (Lesueur)

MEANDRINIDAE
*Meandrina meandrites* (Linnaeus)
*Meandrina braziliensis* (Milne Edwards & Haime)
*Dichocoenia stokesii* Milne Edwards & Haime
*Dendrogyra cylindrus* Ehrenberg

MUSSIDAE
*Mussa angulosa* (Pallas)
*Isophyllastrea rigida* (Dana)
*Isophyllia multiflora* (Verrill)
*Mycetophyllia lamarckana* Milne Edwards & Haime

CARYOPHYLLIIDAE
*Eusmilia fastigiata* (Pallas)

In addition, *Oculina varicosa* and *O. diffusa* were reported from Barbados by Nutting (7), but these were not found on the reefs.

## Description of the Reefs

*Reef Flat Zone*

A characteristic feature of Barbadian reefs is a wide band of nearly flat reef-rock surface lying between the shore and the zones of actively growing coral. It varies in width from 10 to 70 meters and is on the average about 50 meters wide. It is limited on the inner edge by a sand beach and the shifting of the beaches during periods of heavy seas makes this landward limit very unstable. The outer edge is defined by an increasing irregularity of the bottom and by the appearance of the encrusting coral *Diploria clivosa* and the colonial zooanthid *Palythoa mammillosa.*

It is a shallow area with a depth of no more than 1 foot of water at mean low tide at its inner edge and 1 to 3 feet at its outer limit. The inner edge remains barely wetted at low water spring tides. The bottom consists chiefly of reef debris, flat circular stones, loosely cemented calcareous fragments mixed with sand, or a flat rock pavement. The rock pavement is usually covered with a thick algal turf composed of a filamentous green alga which holds and binds fine sand grains, or the surface may be bare with a coating of pink or purple coralline algae.

Three species of algae regularly achieve attachment in this zone, a *Sargassum* sp., a *Padina* sp., and a *Dictyota* sp. They fasten both to the rock pavement and to the surface of the large boulders. In a few places the turtle grass, *Thallasia testudinum,* flourishes but this species is not typical of exposed reef flats.

The only coral which is common on the reef flat is the small encrusting species, *Siderastrea radians.* Scattered colonies, a few inches in diameter, are common on all reef flats. Stunted colonies of the branching *Porites porites* occur occasionally in the *Thallasia* beds.

While corals are poorly represented in the reef flat zone, there is often a rich variety of other invertebrates. Beneath the flattened boulders, large numbers of the ophiuroids, *Ophiocoma echinata* and *O. riisei,* occur. Other common forms are the echinoids, *Tripneustes esculentus* and *Echinometra*

*lucunter;* the burrowing spatangoid, *Brissus brissus;* the holothurians, *Holothuria arenicola, H. parvula,* and *Stichopus badionotus;* the asteroids, *Linckia guildingii* and *Ophidiaster guildingii;* the whelk, *Thais deltoidea;* the hermit crabs, *Erpiphia gonogra* and *Clibanarius tricolor;* several species of porcellanid and grapsoid crabs; and ascidians, Bryozoa, sponges, and polychaetes.

*Diploria–Palythoa Zone*

This zone is characterized by the extensive colonization of two forms, the coral *Diploria clivosa* and the colonial zooanthid *Palythoa mammillosa,* and by the increased irregularity of the bottom topography. Low mounds of reef rock and sharp projections appear at the outer edge of the reef flat zone. The seaward limit of the *Diploria–Polythoa* zone is more irregular and roughened than the landward limit, and the zone is evidently one in which the rock surface is being eroded and flattened.

The zone varies in width from 5 to 20 meters and is, on an average, about 10 meters wide. At its outer limit it is covered by about 2 feet of water at mean low tide. There are many potholes a few feet deep and winding rubble-filled channels which continue outwards into the seaward edge of the reef. The surface of the zone is slightly higher than the reef flat.

*Diploria clivosa* is the most successful coral occurring in this zone. It forms low encrustations 1 or 2 ft wide up to 6 ft in diameter. Towards the outer edge of the zone where potholes and other depressions occur, the rock surface is colonized by half a dozen other corals. *Porites porites* occurs in stunted colonies, and there are flat or rounded encrustations of *Porites astreoides.* There are also rounded boulders of *Diploria strigosa* and scattered colonies of *Favia fragum* and *Montastrea annularis.* On the sides of the potholes, small irregularly rounded colonies of *Agaricia agaricites* are encrusted.

The colonial zooanthid, *Palythoa mammillosa* is very abundant in this zone. Wide sheets, several meters in diameter, are formed or it occurs as small closely adjoining rounded colonies (Fig. 3).

The hydroid *Millepora alcicornis* is often abundant on the rock surface. The colonies are short, stout, and encrusting, or form short blade-like structures. The ubiquitous sea urchin, *Diadema antillarum,* is abundant in holes and crevices. The large anemone, *Stoichactis helianthus,* is abundant on some reefs and may form widespread sheets several meters across. These sheets can exclude the settlement of all other organisms. An encrusting alcyonarian, *Erythropodium caribaeorum,* is often common on the sides of the valleys at the outer edge of the zone.

On all reefs, where the surface is bare of other encrusting forms, the rock is covered by a layer of coralline algae, and small branching clusters of a purple coralline algae are common in crevices.

*Reef Crest Zone*

The reef crest is a region of prolific coral growth and, in terms of development and succession of corals, it is the climax zone of the living reef. The width of the zone varies from 20 to 100 meters. Its seaward limit is defined as the point at which the profile of the reef begins to slope downwards towards

the bottom. Between this point and the outer edge of the previous zone, the reef maintains a more or less horizontal surface 2 to 4 feet below water at mean low tide.

The basic feature of this zone is a series of spurs or ridges which project outwards towards the sea and alternate with irregular winding valleys. The height of these spurs from the bottom is 4 to 6 meters at the outer limit. This formation is similar to that described by Goreau (2) for the buttress zone of Jamaican reefs, and by Newell and Rigby (5) for Andros reefs in the Bahamas. The bottoms of the valleys are composed of sand towards the outer edge, and debris blocks or fragments at the inner edge where the valleys narrow and merge with the reef rock pavement or become choked with debris.

The spurs, which lie perpendicular to the shore, vary from 3 to 7 meters in width and are irregular in outline. They are often bisected across their widths to form separate massive blocks. The sides of the spurs are perpendicular but are undercut at their bases. Towards the outer edge of the zone, the walls are composed chiefly of *Montastrea annularis*, which forms shingle-like plates, simple sheet-like faces, or lobular colonies (Figs. 4, 5, and 6). *Siderastrea siderea* occasionally replaces *Montastrea* as the chief component of the sides of the spurs. It does not, however, form vertical faces but rather large rounded masses which bulge outwards from the walls into the valleys.

From mid-zone to the inner edge of the reef crest, large portions of the walls have broken off due to the undercutting at the base, and here the walls are sloping and irregular. The walls in this part of the zone are encrusted with a variety of other corals which are established after the breakoff of the walls.

The surface of the zone is approximately horizontal but with deep holes and cavities and sharp projections. This horizontal surface is composed entirely of dead coral rock which has become secondarily encrusted with a number of different species of corals (Fig. 7). *Porites porites* and *Porites astreoides* are codominant species. The intensity of colonization increases towards the seaward edge where these two species cover more than 75% of the rock surface.

The following species occur on the horizontal surface:

*Porites porites*
*Porites astreoides*
*Montastrea annularis*
*Montastrea cavernosa*
*Acropora palmata*
*Diploria clivosa*
*Diploria strigosa*
*Diploria labyrinthiformis*
*Favia fragum*
*Siderastrea siderea*

On the walls of the spurs, except at the outer edge, the following species are found:

*Stephanocoenia michelinii*
*Montastrea cavernosa*
*Montastrea annularis*
*Agaricia agaricites*
*Siderastrea siderea*
*Porites astreoides*
*Mycetophyllia lamarckana*
*Isophyllastrea rigida*
*Isophyllia multiflora*
*Eusmilia fastigiata*
*Colpophyllia natans*

On many of the reefs this zone is poorly developed. *Montastrea annularis* is often scarce and the spurs are loosely connected, low, and with sparse

encrustations of other corals. Wide areas have an encrustation of pink coralline algae and scattered colonies of *Porites porites*, but appear otherwise devoid of life.

Of the other invertebrates found in the reef crest zone, the hydroid *Millepora alcicornis* is the most common (Fig. 8). It forms clumps of blade-like structures on the surface and grows up to the water level of low water spring tides. Other common forms include the peculiar little anemone *Hoplophoria coralligens*. This species has two sets of tentacles, one lobate and the other lanceolate. It lives in narrow crevices all over the surface of the rock. The large anemone *Stoichactis helianthus* occurs on the walls of the spurs and on debris at the bottoms of the valleys, and two other species of anemones, *Zooanthus pulchellus* and *Ricordea florida*, are common. The large polychaete *Sabellastarte magnifica* is common and occurs between the branches of *Porites porites*. The gastropod *Coralliophila abbreviatum* is often abundant on partly eroded heads of *Montastrea annularis* and appears to be one of the agents of surface destruction of this coral.

*Seaward Slope*

This zone is characterized by a gradual slope of the reef surface from the crest to the bottom at a depth of 5 to 6 meters. At the outer limit, 30 to 150 meters beyond the reef crest, low ridges appear as outcrops 1 to 3 meters high from a sand or rubble bottom. Elsewhere the ridges may be continuous with the reef crest and extend seaward in a gentle slope to the bottom. Near the reef crest, separate massive boulders, 3 to 5 meters in height and 5 or 6 meters in diameter, appear. The ridges are separated by sand bottom (the seaward continuations of the valleys of the reef crest zone) or may be joined by low growths of massive corals. Valleys are less regular in this zone and they tend to divide and form large open patches of sand bottom.

The dominant coral in this zone is *Montastrea annularis*. It is found not only on the sides of the larger blocks as flat sheets and shingles, but also upon the sides of the low ridges and on top as large clumps of lobular colonies (Fig. 13). *Siderastrea siderea* is also an important structural element of the sides of the crests.

At the inner limit of the seaward slope, the composition of the corals on the ridges is the same as that of the reef crest with several additions. There is a gradual progression outwards, however, to the outer edge where *Porites porites* and another branching form, *Madracis asperula*, become codominants (Fig. 9). At depths of 5 to 6 meters, pure or mixed stands of these two species cover the lower lying ridges.

The following species are common in the seaward slope zone:

*Madracis asperula*
*M. decactis*
*Porites porites*
*P. astreoides*
*Agaricia agaricites*
*Siderastrea siderea*
*Diploria strigosa*
*Diploria labyrinthiformis*
*Colpophyllia natans*
*Colpophyllia amaranthus*
*Montastrea annularis*
*Montastrea cavernosa*
*Dendrogyra cylindrus*
*Mussa angulosa*
*Eusmilia fastigiata*
*Isophyllastrea rigida*
*Mycetophyllia lamarckana*
*Isophyllia multiflora*

*Reef Front*

As the forward edge of the seaward slope decreases in height, the low ridges disappear and are replaced by scattered patches of coral heads. A band of sand and rubble bottom, which is dotted with these patches, lies along most of the leeward coast at depths of about 9 to 10 meters. The density of the patches varies widely from place to place.

The dominant coral of this zone is again *Montastrea annularis*. It forms groups of lobular colonies and is the chief structural base for the settlement of other species of corals. The clumps reach a height of about 4 feet and vary from 2 to 8 feet in diameter.

Associated with *Montastrea annularis* are *Diploria strigosa* and *Diploria labyrinthiformis*, *Porites porites*, *Meandrina meandrites*, *M. braziliensis*, *Dichocoenia stokesii*, *Siderastrea siderea*, *Agaricia agaricites* and *A. fragilis*, and *Porites astreoides*. *Siderastrea siderea* sometimes replaces *Montastrea annularis* as the primary colonizer. *Dichocoenia stokesii* and *Meandrina meandrites* are very successful in this zone although they do not form large colonies. They do not occur in the seaward slope zone and are indicator species for the reef front. *M. meandrites* is able to develop directly upon a sand or rubble bottom.

A number of other invertebrates are characteristically found associated with the scattered clumps. The hydroid *Millepora alcicornis* forms encrustations on many of the clumps, but it is low and stunted and never of the blade-like form. The echinoid *Diadema antillarum* is abundant and the anemones *Aiptasia annulata* and *Condylactis gigantea* are found in nearly every group. Several species of gorgonids are common, and the sponges *Desychalina cyathina*, *Verongia fistularis*, and *Spheciospongia vesperia*. The crustaceans *Stenorynchus seticornis* and *Stenopus hispidus* are often closely associated with *Diadema*, and burrowing in the sand around the base of the rocks is the synaptid holothurian, *Euapta lappa*. The large holothurian, *Holothuria mexicana*, occurs on the surface of the sand.

*Deep Water Communities*

The bottom communities of corals from depths of 10 meters outwards to the limit of the investigations at about 30 meters is an extremely rich one. There are not only extensive mixed stands of corals, but there is a great diversity of species of corals and of other invertebrates. Two types of communities are present; pure stands of *Madracis asperula* (mixed with *Porites porites* at shallower depths), and mixed stands of nearly two dozen coral species.

*Madracis asperula* is normally a deep water species (J. W. Wells, personal communication) and is a slender branching form. It has been dredged from depths of 50 to 100 fathoms locally on several occasions. The shallow water form on the Barbados reefs is, however, a stout-branched form. The branches grow upright and are close together. The polyps are extended during the daytime and the surface of living tissue extends for about 1 inch downwards from the tip of the branch. Below the growing area, the branches are covered

with coralline algae and sponges which bind the dead branches together. In deeper water this base of sponge-bound branches reaches thicknesses of 2 or 3 ft.

Extensive beds of *Madracis asperula* occur as almost pure stands from depths of about 10 meters (Fig. 10). The closely packed branches form a complete covering, interrupted by patches of other corals or *Madracis* rubble to depths of at least 30 meters.

The interstices between dead branches support a rich invertebrate fauna. The ophiuran, *Ophiothrix suensonii*, is abundant on the surface of the coral or between the ends of the branches. Several other species of ophiurans occur deeper within the crevices. Two starfish, *Ophidiaster guildingii* and *Linckia guildingii*, are common, and there is a small synaptid holothurian. There is an abundant microfauna including gastropods, pelecypods, small crabs, annelids, amphipods, isopods, and alpheid shrimp. The unbranched gorgonid, *Briarum asbestinum* is a characteristic feature of the *Madracis* beds as is the large yellow tubular sponge, *Verongia fistularis*. Several species of comatulid crinoids are also common.

Alternating with the *Madracis* beds are stands of mixed corals. In these stands are found more than half the species of corals occurring in Barbados. A list of the species and their relative abundance in the community is shown below.

| Coral species | Abundant | Common | Rare |
|---|---|---|---|
| *Montastrea annularis* | x | | |
| " *cavernosa* | x | | |
| *Siderastrea siderea* | x | | |
| *Diploria strigosa* | | x | |
| " *labyrinthiformis* | | x | |
| *Agaricia agaricites* | | x | |
| " *nobilis* | | x | |
| " *fragilus* | | | x |
| *Acropora cervicornis* | x | | |
| *Mussa angulosa* | | x | |
| *Eusmilia fastigiata* | | x | |
| *Porites porites* | x | | |
| " *astreoides* | | x | |
| " *furcata* | x | | |
| *Madracis asperula* | x | | |
| " *decactis* | | x | |
| *Colpophyllia natans* | | x | |
| " *amaranthus* | | x | |
| *Mycetophyllia lamarckana* | | x | |
| *Isophyllastrea rigida* | | x | |
| *Dichocoenia stokesii* | | | x |
| *Meandrina meandrites* | | x | |
| *Favia fragum* | | x | |
| *Dendrogyra cylindrus* | | | x |

This mixed community alternates with beds of *Madracis asperula*. The community never achieves substantial height, but forms rather a low cover over the bottom. Rounded heads of *M. annularis* reach a height of 1 or 2 ft, while *A. cervicornis* reaches 3 or 4 ft.

In addition to the variety of species present, a striking feature of this community is the intensity of the colonization. The bottom at deeper levels is almost completely covered by living coral. Massive and encrusting species such as *Montastrea*, *Siderastrea*, and *Colpophyllia* grow on the substrate while *Porites* and *Acropora* grow up from crevices and form a branching network above the massive species (Figs. 11 and 12).

The other invertebrate fauna of this mixed community is also rich and varied. Perhaps the most striking animals of the community are the ophiuroids. These occur on almost foothold, clinging to sponges and gorgonids and festooned on the branches of the corals. The glassy spined *Ophiothrix suensonii* is the most common, but several other species occur in the crevices at the base of the corals. The unbranched gorgonid, *Briarium asbestinum*, is very common as are several branching forms. The anemones *Aiptasia annulata* and *Condylactis gigantea* are common. A simple unbranched antipatharian occurs at deeper levels. *Millepora alcicornis* is abundant and forms delicately branching structures as well as knotted encrustations on the branches of dead corals. Several species of comatulid crinoids of the genus *Nemaster* are common.

*Windward Reefs*

No actively growing reefs were found on the eastern coast of Barbados. Most of this coast is fringed with dead reefs which extend outwards from the shore for distances of several hundred meters. At their outer limit they drop sharply to a sand and rubble bottom at 50 to 60 ft. The surface of these reefs is dead and is only sparsely colonized by living encrusting corals.

Extensive reef flats, 100 to 200 meters wide, front the sand beaches and escarpments of the windward coast. These are formed of coral rock. They are covered by about a foot of water at mean low tide and are sometimes exposed along their inner edges at low water spring tides. A heavy cover of attached *Sargassum* sp. is present along the outer edge and the flats are subject to heavy wave action, for at high tide long "rollers" pour over it continuously.

A few species of corals are found on the flats. At the inner edge around the bases of holes and tide pools small colonies of *Porites divaricata*, *Siderastrea radians*, *Favia fragum*, and *Porites astreoides* occur.

Along the outer edge of the flats the water deepens to 8 to 10 ft or drops sharply to about 60 ft. Vertical sides are thus formed which face directly into the prevailing wind. Strong alongshore currents prevail and the water is heavily laden with sediment. Visibility is limited to less than 10 ft during most of the year. The vertical faces are deeply undercut with deep caves penetrating far into the rock and large block and debris falls are found at their bases.

The most characteristic feature of the zone seaward of the reef flat is the complete absence of branching corals. Furthermore, the massive species such as *Montastrea*, *Siderastrea*, and *Diploria* do not form rounded heads but are encrusting and flattened.

At the outer edge of the reef, from depths of 8 or 10 ft to 60 ft, and on the sides of the vertical walls, the following species occur:

| Species | Occurrence | Remarks |
|---|---|---|
| *Porites astreoides* | Common | Low and encrusting, on horizontal surfaces |
| *Montastrea annularis* | Rare | Encrusting, 2–4 ft wide |
| " *cavernosa* | Common | Encrusting on walls |
| *Agaricia agaricites* | Common | Small encrusting knobs or well-developed shingle-like colonies |
| *Siderastrea siderea* | Abundant | Large heads at base of walls |
| *Diploria clivosa* | Common | Broad encrusting sheets or mushroom-shaped heads on a short stout stalk |
| " *strigosa* | Common | Encrusting on vertical walls and horizontal surfaces |
| *Colpophyllia natans* | Common | Encrusting as elsewhere on other reefs |
| *Acropora palmata* | Common | Disk-like colonies on a broad short stalk. On horizontal surfaces at about 10 ft |

Less than 50% of the surface area of the reef rock is covered with living coral. Of the other invertebrate members in the community, *Millepora* forms low, stoutly branching colonies. The sea urchin, *Diadema antillarum*, is conspicuously absent. There are a number of alcyonarians present of which the commonest is the sea fan, *Rhipidigorgia flabellum*. It forms small colonies with a broad base.

## Development of a Reef

The massive coral, *M. annularis*, is the dominant reef coral of local reefs and is the chief structural element. Its importance as a reef builder in the West Indies has been noted by Smith (10) and by Newell (4). In Jamaica, Goreau (2) found that *M. annularis* forms the framework base of living reefs and is the chief element of the seaward growth of the reefs. In Florida, *Acropora palmata* has been reported as the primary structural element of the reef mass by Ginsburg (1). Newell *et al.* (6) reported *A. palmata* as the most conspicuous species of Andros reefs in the Bahamas, but they state that it is replaced on the seaward edge by massive corals including *M. annularis*. Reefs seen by the author in St. Vincent, W.I., and in Puerto Rico had conspicuous growths of *Acropora* on the seaward edges. There were, however, large blocks of *M. annularis* mixed with *A. palmata* in both areas.

In Barbados, the first stage of reef growth takes place in the area of the reef front and the seaward edge of the reef slope. Widely scattered colonies of *M. annularis*, *S. siderea*, and other massive corals are established on the sand and rubble bottom. By their continued growth and decay, they increase the proportion of rubble and hard rock of the bottom and patches of dead and living corals are thus developed. These patches, a few feet in height, develop in the outer edge of the seaward slope into long winding valleys, carved by the shifting sand that moves in and out of the valleys of the reef crest zone. Settlement of *P. porites* and *M. asperula* takes place on these ridges at an early time and they contribute substantially to the basal framework of the ridges.

The second stage is the establishment of clumps of pure stands of *M. annularis* (Fig. 13). In the seaward slope zone, low clumps of hemispheroidal colonies 1 to 3 meters in diameter are formed and displace the other corals. They colonize the surface and sides of the low ridges and are able to develop to a limited degree on rubble bottom between the ridges.

These clumps are more or less rounded in shape and the greater portion of the tops and sides are living. During the early stages of their growth, they expand in all directions, upwards and outwards from a central focus. The colonies themselves become elongated so that each hemisphere forms a cap of living tissue on top of a long column of dead skeleton. The central colonies of the clumps maintain a horizontal surface while the colonies around the periphery develop a vertical surface, i.e., the axis of outward growth lies at 90° to the central colonies. Thus, during the initial stages of growth, the clumps expand in size as an expanding hemisphere.

Two important changes take place in the clumps as they expand. First, the crevasses between colonies become filled with silt and other water-borne debris. In addition, the sides of the columns which are dead, provide a foothold for the colonization of other corals, algae, and a variety of encrusting forms (Fig. 14).

Secondly, around the periphery of the clumps, where there is less opportunity for catchment and settling, the colonies begin to coalesce and a sheet of living coral forms around the bases of the clumps.

The mid-region of the seaward slope zone is dominated by these low clumps of *M. annularis* which reach a height of 1 or 2 meters. As they reach a diameter of 3 or 4 meters, fusion of the clumps takes place along the length of the ridges. At this stage of reef development, the heights of the ridges, including the clumps of *Montastrea*, average about 3 to 4 meters.

In the next stage there is further establishment of secondary growth corals and the choking out and organic destruction of the central colonies of the clumps. Wide areas of dead colonies form in the centers and there is intensive colonization by *P. porites* and *A. agaricites*. Furthermore, the increasing height of the colonies, which at this stage may reach 3 meters, makes the clumps unstable and there is a tendency for colonies to be broken off (Fig. 15). A great deal of destruction takes place during this stage and about 50% of the clumps never achieve larger size. A good deal of debris is collected around the base of the ridges, to be partly buried and colonized or to be carried inwards along the valleys to the inner zones of the reef. Exposure of new settling surfaces caused by the loss of portions of the clumps allows intensive colonization by other corals, and a large part of the inner region of the seaward slope is built by the accumulation of these additional species.

In those clumps which have resisted destruction most of the centers are dead and encrusted with secondary colonizers. *M. annularis* remains living around the edge as irregular sheets and around the periphery of the tops as hemispheroidal colonies. Near the bases of the clumps there is a tendency for the sheets to grow downwards and outwards over any irregularities or

dead areas and shingle-like colonies develop. The three forms are thus a function of age and position on the mature clump.

The inner region of the seaward slope is dominated by these large irregular clumps or blocks of *M. annularis* which reach a height of 3 or 4 meters with vertical sides.

The final stages of the development of the reef which terminate in the formation of the massive blocks of the seaward slope and the buttresses of the reef crest consist of the further upward growth of *M. annularis* and colonization by other corals. The clumps of *M. annularis* have formed principally along the ridges and are more or less aligned. These have merged as they expanded laterally and become continuous with the ridges of the reef crest. Growth of *Montastrea* continues along the tops and sides of the ridges but, because of the widespread destruction of these clumps, much of the increased height of the frontal ridges is produced by the accumulation of secondary colonizers such as *P. porites*, *P. astreoides*, *A. agaricites*, *D. strigosa*, *D. labyrinthiformis*, *M. cavernosa*, and *S. siderea*.

The massive blocks of the inner portion of the seaward slope are produced by the growth and maturation of one or several clumps of *M. annularis* which have resisted destruction. The walls of these blocks are composed of sheets and shingles of *Montastrea* while the tops are a mixture of a number of corals. These blocks produce the well-formed buttresses of the reef crest.

## Acknowledgment

I am indebted to Dr. John W. Wells of Cornell University for identification of a collection of corals, to Dr. Charles E. Cutress of the U.S. National Museum for his identification of anemones and to Dr. Frederick M. Bayer of the U.S. National Museum for the identification of alcyonarians. Nomenclature of the species is based on the work of Vaughan and Wells (13).

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## EXPLANATION OF FIGURES

FIG. 2. Aerial photograph of a portion of the western coast of Barbados showing reef formations.

FIG. 3. *Palythoa mammillosa* in the Palythoa–Diploria zone.

FIG. 4. Shingle form of *Montastrea annularis* in the reef crest zone.

FIG. 5. Sheet form of *Montastrea annularis* in the reef crest zone.

FIG. 6. Rounded colonies of *Montastrea annularis* in the reef crest zone.

FIG. 7. *Porites porites* and *Porites astreoides* in the reef crest zone.

FIG. 8. Blade-like form of *Millepora alcicornis* in the reef crest zone.

FIG. 9. Mixed *Porites porites* and *Madracis asperula* in the seaward slope zone.

FIG. 10. Pure stand of *Madracis asperula* at a depth of 20 meters.

FIG. 11. Mixed community of *Madracis asperula* and *Acropora cervicornis* with the alcyenarian *Briareum asbestinum* at a depth of 20 meters.

FIG. 12. Mixed community of *Montastrea annularis*, *Montastrea cavernosa*, *Acropora cervicornis*, *Siderastrea siderea*, *Porites astreoides*, *Colpophyllia natans*, and *Mycetophyllia lamarckana* at a depth of 20 meters.

FIG. 13. Pure stand of *Montastrea annularis* in the seaward slope zone.

FIG. 14. Clump of *Montastrea annularis* showing dead center and secondary settlement by *Porites porites*.

FIG. 15. Clump of *Montastrea annularis* showing columns broken off.

NOTE: Figs. 2–15 follow.

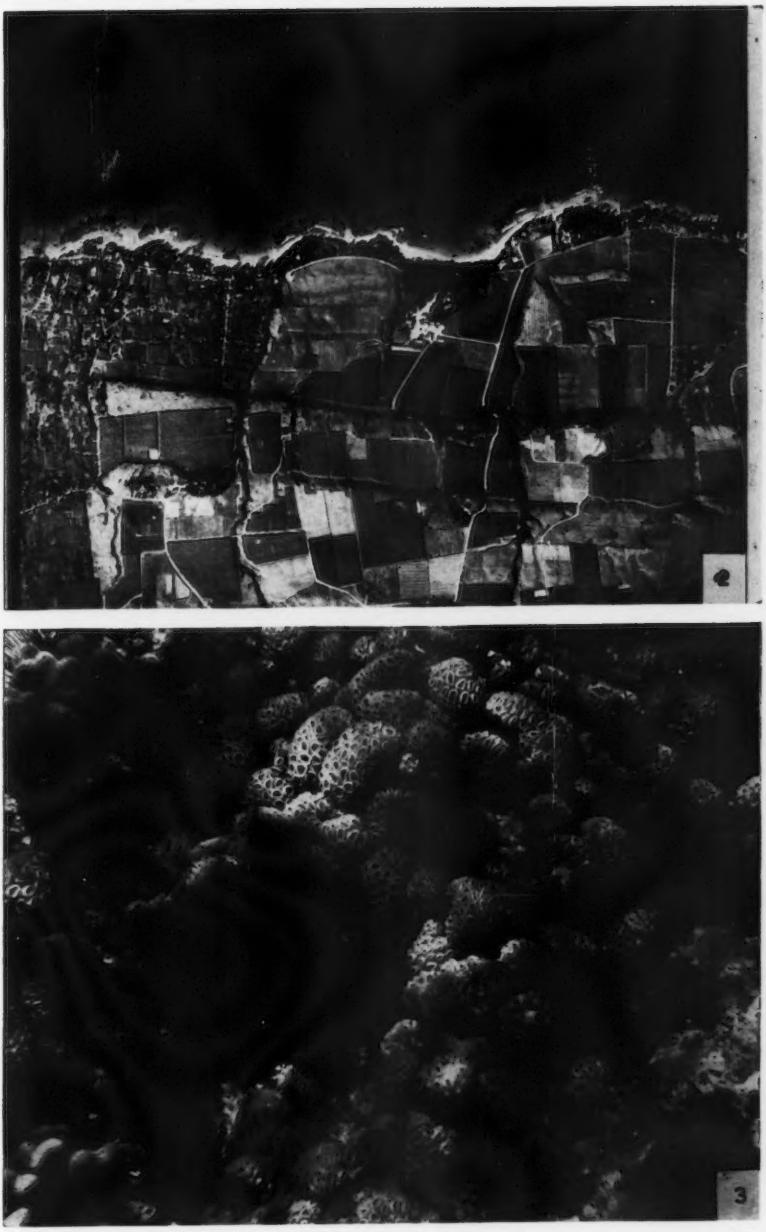
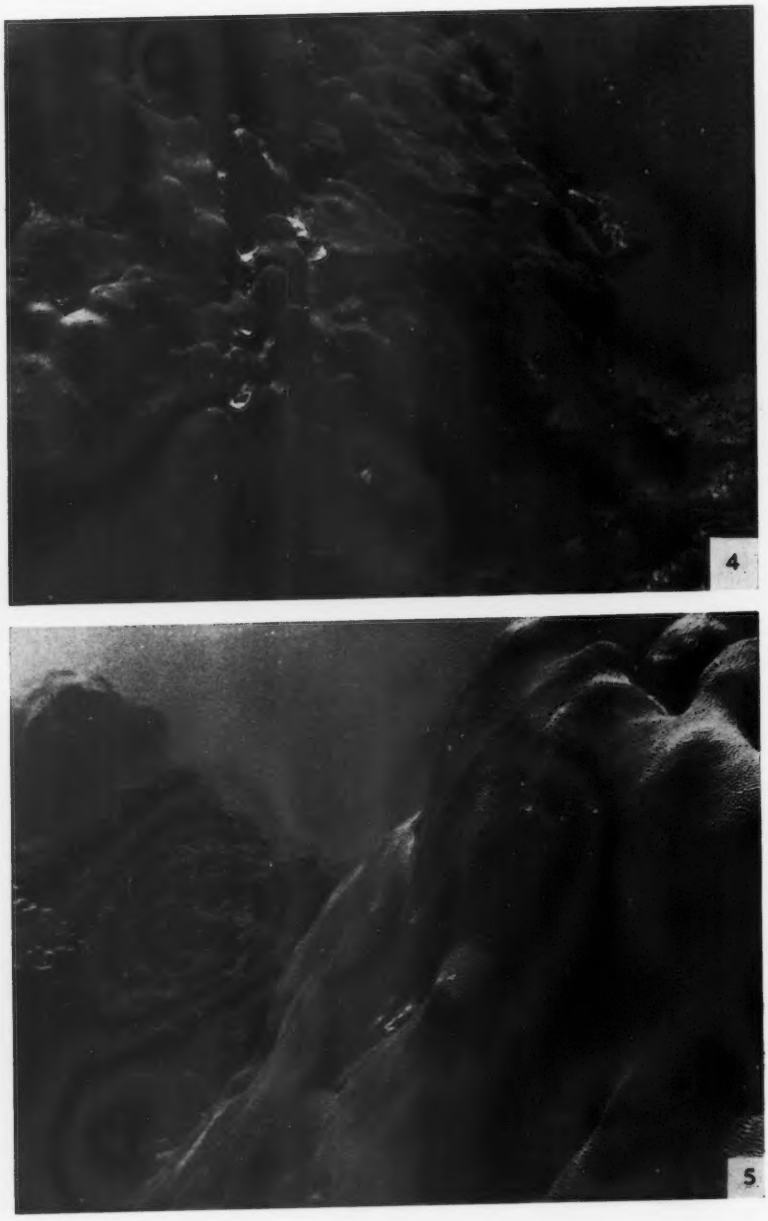


PLATE II



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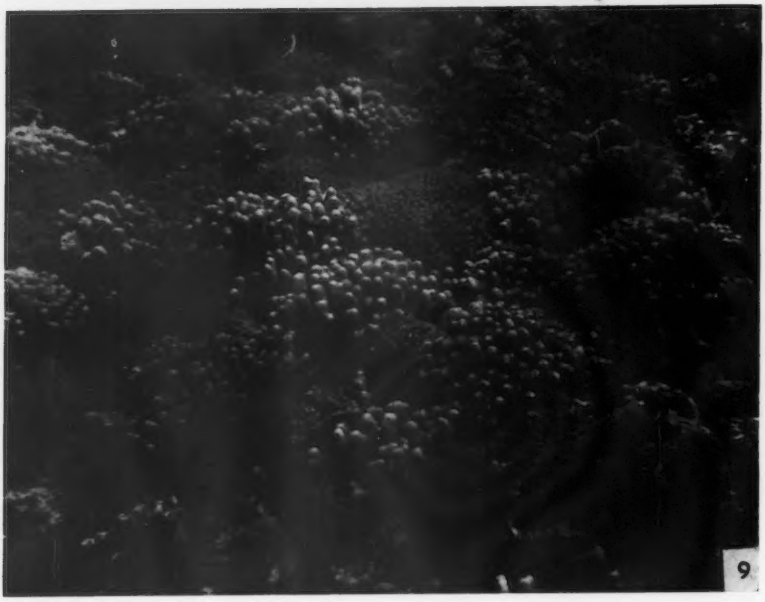
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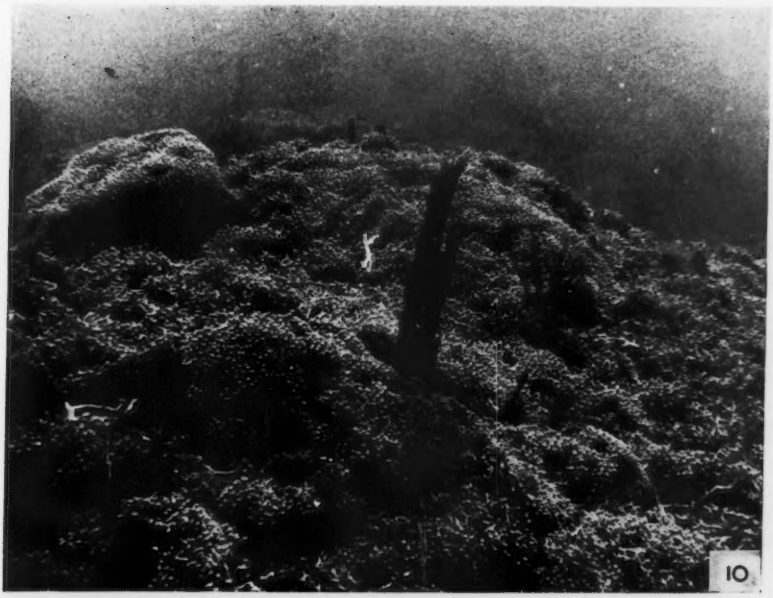
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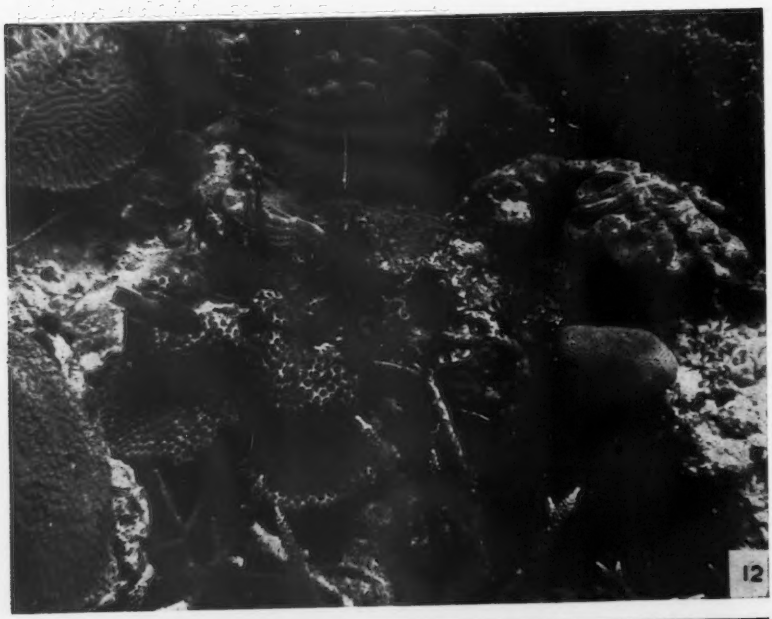

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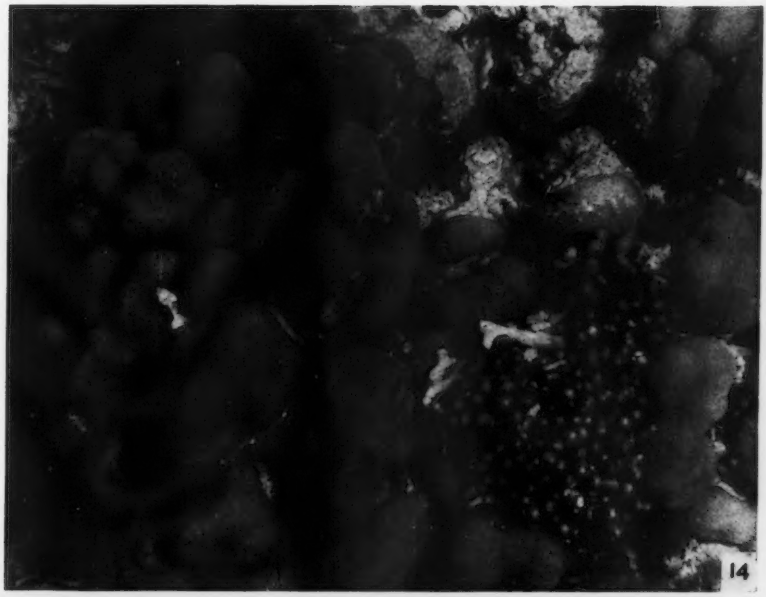
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# THE EFFECT OF DDT ON OVIPOSITION, AND ON BEHAVIOR, IN THE EUROPEAN RED MITE, PANONYCHUS ULMI (KOCH)[1]

D. P. PIELOU

## Abstract

It has not been possible to confirm the statement that DDT brings about increased egg production in the European red mite, *Panonychus ulmi* (Koch). Newly emerged females were allowed to oviposit individually on single, whole, isolated apple leaves for their entire lifetime. On leaves sprayed with DDT, with mean deposits of 3.1 and 10.6 μg per sq. cm, the total egg production per female, the length of life, and the daily rate of oviposition were not significantly different from those on nonsprayed leaves. Females were also placed on isolated leaves, one-half of which had been sprayed with DDT. These females subsequently spent 3.4 times as much time on the unsprayed halves of the leaves as on the sprayed halves; they laid 5.6 times more eggs on the former than on the latter. This species, therefore, is evidently irritated and repelled by DDT.

## Introduction

Increase of the European red mite, *Panonychus ulmi* (Koch), following application of DDT (2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane) for the control of the codling moth, *Carpocapsa pomonella* (L.), has been a world-wide phenomenon. Though destruction of predators of the mite, by the DDT, has generally been considered the cause of the increase, field evidence has gradually accumulated, particularly from western North America, that other factors are involved (1, 6, 12, and 13). Hueck *et al.* (5) have asserted that DDT, in Holland, has a direct effect in the stimulation of increased oviposition in this species of mite. However, in another mite, *Tetranychus multisetis* McGregor, DDT decreases both egg production and longevity, and here there is also evidence that DDT has an irritant, or repellent, effect that, in some way, brings about an eventual increase of the population (2).

The need for confirmation of the stimulating effect in *P. ulmi* has been stressed (13). In Ontario, Herne (4), working with *Tetranychus telarius* (L.), was unable to demonstrate any increase in egg production either through a direct effect, or through a change brought about in the host plant by DDT.

In the work reported here an attempt is made to confirm, or deny, the existence of an increase in egg-laying, and a repellent or irritant effect, in a strain of *P. ulmi* from the Okanagan Valley of British Columbia.

## Methods

The mites were observed on single leaves of apple (variety, Starking Red Delicious) placed with their undersides in contact with a bed of sodden cotton in a petri dish. Mites are thus confined to the upper surface of the leaf by the water barrier in the petri dish. This has been shown to be an effective method of studying orchard mites (8). Under these circumstances, apple

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leaves, provided they are not cut nor broken, last 6 weeks before showing appreciable rotting; small cut pieces of apple leaf, however, decay rapidly. Particular care was taken to choose satisfactory and undamaged leaves. The leaves had an average area of approximately 30 sq. cm. The stock of mites had originally been obtained from a tree in an orchard where DDT spraying had been practised for at least 10 years.

In the greenhouse, overwintering eggs from the orchard were placed on dwarf apple trees (Malling IX rootstock) growing in pots. Experiments were conducted with females of the second and third generation derived from these eggs. One quiescent female deutonymph was placed on each leaf. Males were present in half of the oviposition experiments. When no males were present the females laid unfertilized (male-producing) eggs. The eggs were counted under a 10× stereoscopic microscope. To simplify the counting, the eggs were destroyed with a needle as they were recorded. Counts were made daily until the female mites had died. A DeVilbiss atomizer operated from a pressure-stabilized source of compressed air at 20 lb per sq. in. was used to spray the leaves with DDT, 50% wettable powder, at 0.3 and 1.0 g per liter. Spraying was continued for 20 seconds to the upper sides of the leaves, with the leaves held 30 in. from the spray nozzle. Under these conditions the DDT deposits averaged 3.1 and 10.6 μg per sq. cm for the two concentrations that were used. These values approximate the range for fresh deposits encountered in the orchard with efficient concentrate spraying at recommended dosages (14).

## Results

*Oviposition Rate*

Female deutonymphs were set out in petri dish units on unsprayed leaves and on leaves sprayed with the two concentrations of DDT. There were 60 replicates in each case. The whole experiment was repeated in a second series in which both a male mite and a female mite were placed on each leaf. The results of the experiments are summarized in Table I.

In calculating the mean daily rate of egg-laying, a preoviposition period of 2.5 days (9) was deducted from the mean length of life of the female mites.

TABLE I

Egg production and longevity in females of *P. ulmi* exposed to various concentrations of DDT

| | Average deposit of DDT, μg per sq. cm | | | | | |
|---|---|---|---|---|---|---|
| | None | | 3.1 | | 10.6 | |
| Item | Unmated | Mated | Unmated | Mated | Unmated | Mated |
| Mean length of life, days | 23.2 | 24.8 | 21.9 | 22.7 | 22.5 | 24.0 |
| Mean total eggs per lifetime | 61.5 | 63.1 | 59.4 | 60.1 | 62.0 | 59.2 |
| Mean daily rate of egg-laying* | 2.94 | 2.83 | 3.06 | 2.96 | 3.01 | 2.76 |

*Calculated on the basis of a preoviposition period of 2.5 days.

The data on longevity, and on total egg production, were obtained from females that appeared to die from natural causes. In some cases mites wandered off the leaves and drowned in the water. Such individuals were not included in Table I. No significant differences were apparent as a result of DDT application.

*Repellent and Irritant Effect*

In this series of experiments only one-half of a leaf was sprayed with DDT. This was achieved by placing a mask on the leaf, with the edge of the mask lying along the midrib, and then spraying as already described. Only the higher concentration of DDT was used. Sixty petri dish units were set up. Three female deutonymphs were placed on each leaf. In the 10 days subsequent to emergence the mites were observed daily. The positions of the adults on the leaves (i.e., on the sprayed or unsprayed halves) were noted each day. The eggs laid on each half were counted. The results are summarized in Table II.

TABLE II

Response of female *P. ulmi* where offered alternatives of DDT-sprayed and DDT-free leaf surfaces

| | % of records on halves of leaves with: | | Ratio: no DDT/DDT |
|---|---|---|---|
| | No DDT | DDT | |
| Whereabouts of females | 77.3 | 22.7 | 3.40 |
| Eggs laid | 84.8 | 15.2 | 5.58 |

The females spent 3.40 times as long on the unsprayed halves of the leaves as on the sprayed. They laid 5.58 times more eggs on the former than on the latter. Both of these ratios are significantly different from an even distribution. DDT was, therefore, clearly repellent. Its repellence is further emphasized by the fact that, in proportion to the time they spent on the two halves of the leaves, the female mites laid 1.64 (i.e., 5.58/3.40) times as many eggs on the unsprayed halves of the leaves as they did on the sprayed halves.

This withholding, when the mites were on the sprayed part of the leaf, might be described as an irritant effect. It must be concluded, however, that, on a uniformly sprayed leaf where no alternate choice is available to the mites, the mites adapt themselves to the condition and oviposition will return to the normal rate. The findings in Table I taken in conjunction with those in Table II can only be interpreted in this way. Such a mechanism, involving adaptation to a basal rate, when a choice of two alternatives is available, has a formal relation with that described as *klinokinesis* by behavior physiologists (3).

## Discussion

In the work reported here no evidence was obtained to support the view of Hueck *et al.* (5) that DDT can bring about increased oviposition in *P. ulmi*. Most of the evidence presented by these authors is circumstantial.

However, they carried out definitive experiments in which *P. ulmi* females were allowed to oviposit on disks of apple leaf that had been treated with DDT, not as a 50% wettable powder, but as a 5% dust.

It is suggested that the results of Hueck *et al.* (5) were influenced by unusual experimental conditions. As many as 10 female mites were crowded on a leaf-disk with an area of only 3.77 sq. cm. Such disks, as the authors admit, decay rapidly. It is not surprising that, in their control experiments, females had a mean life of only 4.5 days, and an average daily egg production of 0.81.

By contrast, in the experiments reported here, with single females each isolated on an entire leaf of approximately 30 sq. cm area, the corresponding figures were 23.2 days and 2.94 eggs (Table I). These differences are almost certainly related to the fact that the area of leaf per mite was about 80 times greater than in the work of Hueck *et al.* With DDT, the highest rate of daily egg production recorded by these authors was 1.70, much lower than the rates recorded by us (Table I). Their highest rate of egg production and greatest longevity are also lower than those recorded by Newcomer and Yothers (9) 30 years ago, before DDT was known, and those obtained more recently by Parent and Beaulieu (10).

The conclusion that DDT does not influence rate of egg production in *P. ulmi* agrees with that obtained by Herne (4) with *T. telarius* (L.). Davis (2), working with *T. multisetis* McGregor, actually obtained a decrease in egg production following treatment with DDT.

We have confirmed the opinion of Herne (4) and of Davis (2) that DDT has a repellent, or irritant, effect on mites in definitive experiments, with *P. ulmi*. The exact way in which such an effect can lead to an increase in mite population is still conjectural. However, since *P. ulmi* is known to have a contagious distribution on apple (11), a repellent chemical applied to foliage will tend to spread the mites throughout the tree in a more uniform distribution. This, however, would only lead to an increase in mite population if density-dependent factors limited increase in the original contagious distribution. At high densities such factors would certainly operate to limit increase but whether they would, or not, at moderate or low densities, is not yet known.

There is some circumstantial evidence that the repellent effect does, in fact, spread mites throughout a tree in the orchard. Even with the most efficient spraying no large apple tree is ever uniformly sprayed with DDT. Analysis almost invariably shows (14) lesser deposits in the upper regions of foliage of the tree. The repellent effect of DDT should, then, show itself in an increased concentration of mites in the upper parts of the tree. There is quantitative evidence that such stratification does, in fact, occur (7). However, it cannot yet be assumed that this is the result of DDT application; for it is not known if such stratification is absent in unsprayed trees, because populations of mites are always too low there to allow a comparable quantitative study.

The factors that bring about an increase in population of *P. ulmi*, as a result of the application of DDT, are still only partly understood.

## Acknowledgments

I am indebted to Mr. C. V. G. Morgan for a great deal of advice on the rearing and handling of *P. ulmi*, and also to Mr. N. H. Anderson. I am indebted to Mr. K. Williams for carrying out the DDT analyses mentioned in this paper.

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# A PRELIMINARY SYSTEMATIC STUDY OF THE NORTH AMERICAN LARVAE OF THE CADDISFLY FAMILY PHRYGANEIDAE (TRICHOPTERA)[1]

GLENN B. WIGGINS

## Abstract

Twenty-six species of the caddisfly family Phryganeidae are known to occur in a wide variety of aquatic habitats over the northern and montane areas of North America. Larvae are described for 13 of these species, including 5 not previously known. Using data on habitat and various aspects of behavior, along with new structural characters, progress is made in giving broader biological significance to the generic groupings. A key to seven of the eight North American genera is provided, along with keys to the known larvae of the genera *Agrypnia* and *Banksiola*. The general distribution of each species treated is summarized.

As part of my studies on the systematics of the Phryganeidae, a good deal of information has now been gathered concerning the larvae and pupae. Originally, it was my intention to bring this together in a single work on the immature stages of this family, but I have been forced to abandon this plan for the present, partly because progress in the discovery of the unknown immature stages of the North American species has been slow, and partly because I have been unable to obtain sufficient material of the European and Asian species. This paper, then, presents the systematic data that have been brought together for the larvae now known of the North American phryganeids. It must be remembered that the larvae and pupae of 12 of the 26 North American species are as yet undiscovered, so that this study is to some extent provisional, at the generic level, as well as preliminary. For the present, the aim is to facilitate the identification of as many of the larvae as are now known, and to make a beginning, at least, in giving the generic groups broader biological significance by combining data now available on behavior, ecology, and morphology. When the immature stages of more of the species become known, I hope to be able to enlarge this preliminary study of the larvae into a more extensive systematic work on the immature stages of the Phryganeidae.

It is perhaps useful to begin here by outlining the criteria by which a caddisfly larva is assigned to the family Phryganeidae: antennae short and peg-like, situated close to the anterior margin of the head capsule; pronotum completely covered with a broad, heavily sclerotized plate, which bears a transverse furrow or fold a short distance in front of the posterior margin; prosternal horn present; mesonotum usually entirely membranous, but sometimes with a pair of small anteromesal sclerites; metanotum entirely membranous; a small sclerotized knob bearing a cluster of stout hairs present on each side of the mesonotum and metanotum; larval case usually constructed of plant pieces fastened together to make a smooth cylinder.

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Contribution from the Department of Invertebrates, Royal Ontario Museum, Toronto 5, Canada.

## Discussion of Certain of the Characters Used in This Study

*Coxal Combs*

One set of characters here introduced into the taxonomy of phryganeid larvae is the comb-like plates, referred to here as *coxal combs*, on the poster-oventral surface of each coxa. Each comb consists of a series of pointed

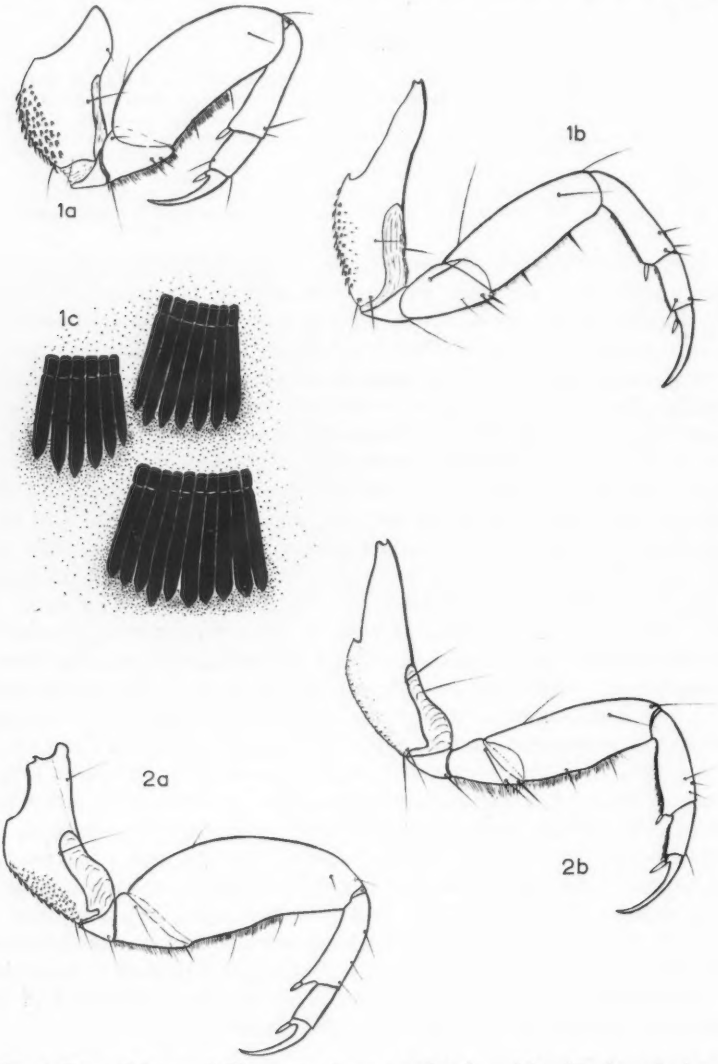



FIG. 1. Legs of larva of *Phryganea cinerea*: *a*, first leg, right side, lateral view; *b*, second leg, right side, lateral view; *c*, coxal combs of first leg, much enlarged. FIG. 2. Legs of larva of *Agrypnia improba*: *a*, first leg, right side, lateral view; *b*, second leg, right side, lateral view.

sclerotized rods (Fig. 1), variable in number and fastened together basally, but separate and somewhat divergent apically. The basal portion of the comb is joined to the coxa, while the apical portion projects from its surface at an angle. Silfvenius (9, 10, 11,) in his descriptions of many of the phryganeid larvae of Finland, calls them *Spitzenkämme*, and various other authors have commented on them. Vorhies (13) refers to them as small spines. Lloyd (3) shows them quite clearly in his figures of the first and second legs of *Phryganea interrupta* (= *sayi*). These coxal combs are present on all of the phryganeid larvae that I have examined. They are variously developed on the first and second legs in different genera, while on the third legs in all genera the combs are reduced and appear as small uniform points under moderate magnification.

In the two genera in which they are best developed, *Phryganea* and *Agrypnia*, the coxal combs are usually largest along the extreme posteroventral edge of the coxa, with smaller combs on each side of this central patch. The sections of the key involving the coxal combs refer to this most strongly developed patch in each case. In *Phryganea* (Fig. 1) the coxal combs of both the first and second pairs of legs are very strongly developed, projecting prominently from the surface of the coxa, somewhat like scales. In *Agrypnia* (Fig. 2) the coxal combs are usually well developed on the first pair of legs, where they project like scales much as in *Phryganea*. On the second legs, however, the combs are much smaller and they do not project as prominently as on the first pair, resembling more the teeth of a file. In one species, *Agrypnia vestita*, the coxal combs of the first legs (Fig. 3) are somewhat smaller than in other species of the genus, although these combs are still larger than in the remaining genera. In *Banksiola* there are coxal combs on the first and second pairs of legs but they are extremely small and their comb-like structure is recognizable only under magnification of around 450× or more. Under moderate binocular magnification of 50× or 100× these combs appear as tiny raised points (Fig. 4). The coxal combs in *Oligotricha* are about the same as in *Banksiola*, although I have seen only the European species *O. ruficrus*. The coxal combs on the first and second legs of *Oligostomis* and *Ptilostomis* are small, about the

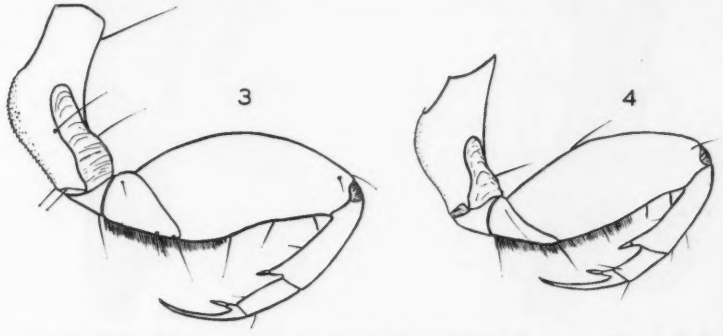



FIG. 3. First leg of larva of *Agrypnia vestita*, right side, lateral view. FIG. 4. First leg of larva of *Banksiola crotchi*, right side, lateral view.

same as in *Banksiola*, and although I have not seen larvae of *Hagenella*, it is very likely that the combs are small there, too. As far as I know, the function of these coxal combs is unknown, although they bear a remarkable resemblance to the tibial combs of many larvae of Odonata, which are there believed to make the legs more effective as cleaning organs (Gardner (2)).

*Color Pattern of the Head and Thorax*

The heads of phryganeid larvae are usually yellow in base color, although this may range from light tan to brownish orange in different species. The very characteristic feature of the head markings in this family is the pair of dark-brown bands that extend over the dorsal surface of the head, referred to here as the *dorsal bands*. These dorsal bands originate at the anterolateral corners of the head capsule, near the antennae and the bases of the mandibles, and converging posteriorly, but laterad of the frontoclypeal sutures, they extend to the posteromesal margin of the head capsule where they almost meet. The dorsal bands vary considerably in width. In some species (Figs. 7, 8, 9) the frontoclypeus bears a median dark-brown band which varies in

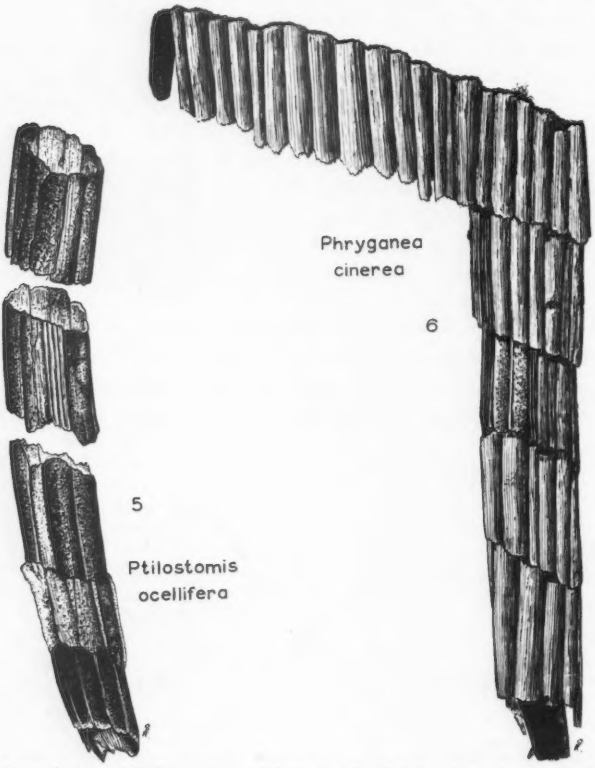
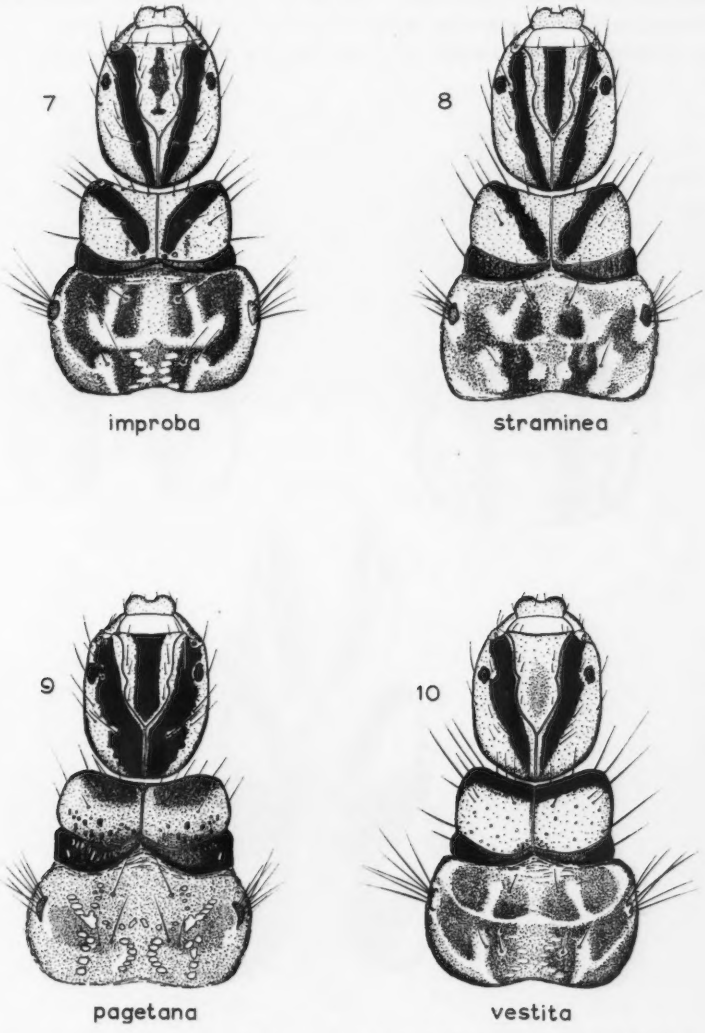


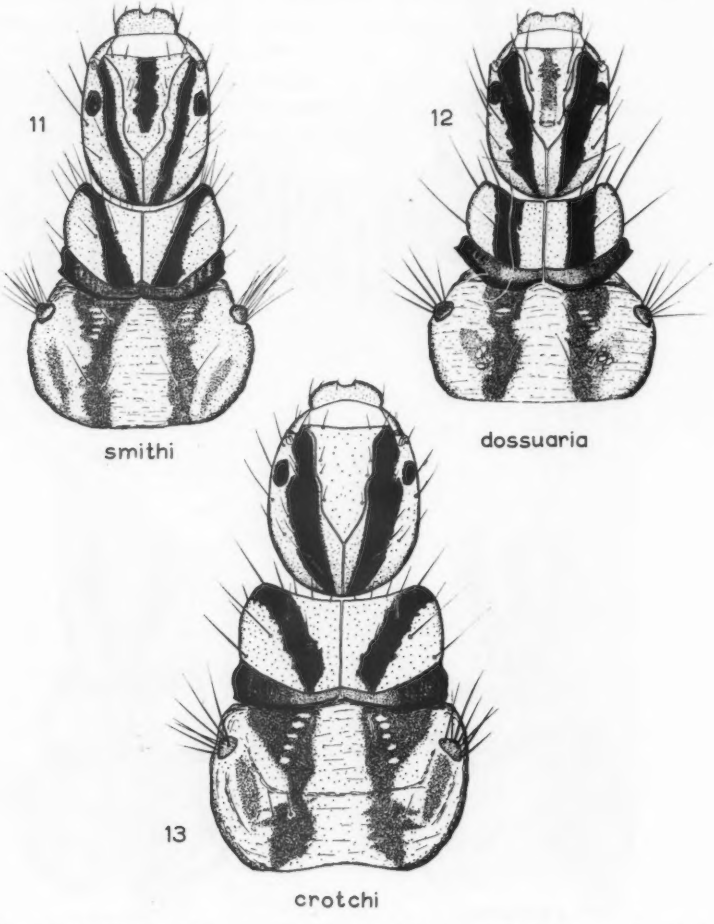

FIG. 5. Larval case of *Ptilostomis ocellifera*, partly disjointed to show cylinder type of construction. FIG. 6. Larval case of *Phryganea cinerea*, partly unwound to show spiral type of construction.

length, width, and intensity of color among different species. The lateral surface of the head in phryganeid larvae also bears a dark-brown band on each side, which in all the North American species now known extends from a point close to the edge of the occipital foramen, almost to the eye. These bands are here referred to as the *lateral bands*. The ventral surface of the head has quite characteristic brown markings in many species; while in others, it is entirely without markings.




FIGS. 7–10. Head, pronotum, and mesonotum of larvae of *Agrypnia* spp., dorsal view. 7, *A. improba*. 8, *A. straminea*. 9, *A. pagetana*. 10, *A. vestita*.

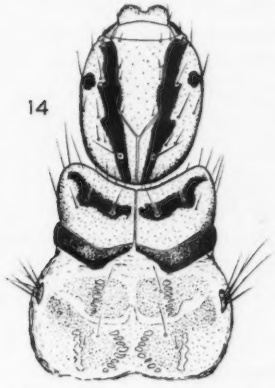
The sclerotized pronotum is usually the same base color as the head and, again like the head, is traversed by dark-brown bands or some other brown markings which vary widely in different species. Certain features of the pronotum are, however, common to all phryganeids. One of these is the prominent furrow, here referred to as the *pronotal furrow*, which lies a short distance in front of the posterior edge of the pronotum, and runs largely parallel to this posterior margin for the whole width of the plate. Another feature common to all phryganeids is the heavy black line bordering the entire posterior margin of the pronotum, terminating at each posterolateral corner at the point where the pronotal furrow reaches the lateral edge of the plate. The relatively narrow



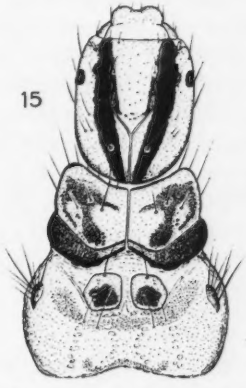

FIGS. 11–13. Head, pronotum, and mesonotum of larvae of *Banksiola* spp., dorsal view. 11, *B. smithi*. 12, *B. dossuaria*. 13, *B. crotchi*.

band between the pronotal furrow and the posterior black line is usually mostly dark brown in color.

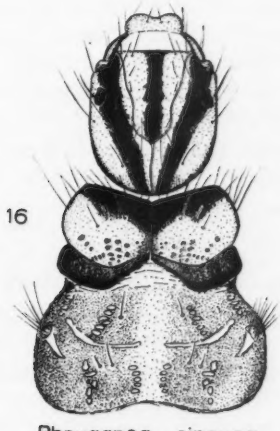
The variations in the pigmentation of the membranous mesonotum and metanotum are also very useful taxonomic characters. Specimens belonging to the genera *Banksiola* (Figs. 11, 12, 13) and *Oligotricha* can be readily recognized by the two dark, irregular bands which extend longitudinally over the last two thoracic segments. These bands are continuous for the most part within the confines of each of the segments, but there are usually several small, unpigmented, ovoid lacunae in each band. The condition in several species of *Agrypnia*, on the other hand, is just the reverse of this. Here, the rather dark




Ptilostomis ocellifera




Oligostomis pardalis




Phryganea cinerea

FIGS. 14–16. Head, pronotum, and mesonotum of larvae, dorsal view. 14, *Ptilostomis ocellifera*. 15, *Oligostomis pardalis*. 16, *Phryganea cinerea*.

color of the mesonotum and metanotum is broken into several blocks by the intervention of unpigmented areas, with the resulting appearance of a pair of longitudinal light bands on a dark background (Figs. 7, 10). In at least one species, *A. pagetana* (Fig. 9), the unpigmented areas are reduced to slender bands, and occasionally some specimens of *Agrypnia* show very little evidence of any pigmentation. In *Phryganea* (Fig. 16) and *Ptilostomis* (Fig. 14) the generally dark color of the mesonotum and metanotum is broken by a pair of narrow, unpigmented longitudinal bands, and this pair is intersected by a narrow, transverse, arcuate unpigmented band. Between the longitudinal unpigmented bands, and behind the transverse band, lies a pair of short, crescent-like, unpigmented bands. In *Oligostomis* (Fig. 15) there is very little color pattern on the mesonotum and metanotum, although with close scrutiny the same basic unpigmented bands that occur in *Phryganea* can be made out. There is, in fact, a basic pattern in these unpigmented bands on the mesonotum and metanotum of all the larvae I have seen belonging to the genera *Phryganea*, *Ptilostomis*, *Oligostomis*, and *Agrypnia*. Certain species of *Agrypnia*, such as *A. improba* (Fig. 7) and *A. vestita* (Fig. 10), differ only in having the unpigmented bands wider, with the separate ovoid unpigmented areas coalesced into a continuous band. The basic pigmentation pattern of the mesonotum and metanotum shared by these genera is quite distinct from the dark bands of *Banksiola* and *Oligotricha*.

*Abdominal Gills*

Differences in the arrangement of the abdominal gills are often of specific significance, and all of these are variants of the basic or complete complement as found in *Phryganea*. Here there are two ventral gills on each side of the first abdominal segment, six on each side of the succeeding abdominal segments up to, and including, the seventh. The six gills on each side of the segments are arranged in two vertical rows of three, and individual gills can thus be clearly designated as either antero- or postero-dorsal, lateral, or ventral. Only the three anterior gills are present on each side of the eighth abdominal segment in *Phryganea*. There is some intraspecific variation in these gills even when the larvae are fully grown, but in larvae that are not fully grown many gills of the normal complement of the mature larva of the species are not present.

*Behavior*

The portable cases constructed by the North American larvae of the Phryganeidae are composed of pieces of plant material fastened together to make a smooth cylinder in two distinct ways. By one method, larvae of certain genera construct a spiral case, composed of a single spiral band of plant pieces placed side by side (Fig. 6). In at least one species, *Phryganea cinerea*, the spiral can be either dextral or sinistral. By the other method, larvae of certain genera construct a case composed of a series of separate rings or short cylinders of plant pieces, the rings joined end to end (Fig. 5).

The larval habit of spinning an anterior sieve membrane of silk before pupation has been discussed previously (Wiggins (15)). Information concerning this habit in various genera and species is added here whenever it is known.

*Habitat*

Phryganeid larvae live in a wide variety of aquatic habitats ranging from cool, spring streams to ponds, lakes, and large rivers. These are always of a permanent nature because caddisflies of this family apparently do not have the ability to live in annually temporary ponds as do certain species of the Limnephilidae.

*Pupal Mandibles*

Although this is primarily a study of larvae, the widely divergent structure of the pupal mandibles in the Phryganeidae appears to be related to the larval habit of spinning an anterior sieve membrane (Wiggins (15)). The type of pupal mandibles known for each genus is thus noted.

## Provisional Generic Key to the Known Larvae of the North American Phryganeidae[2]

1. Coxal combs of the first pair of legs well developed and prominent, often appearing as darkened ridges, the comb-like structure clearly evident (Figs. 1*a*, 2*a*)..... 2
   Coxal combs of the first pair of legs very small, barely visible or appearing as tiny raised points (Fig. 4)..... 3

2. Coxal combs of the second pair of legs large and well developed, projecting prominently from the posteroventral surface of the coxa (Fig. 1*b*), similar to the combs on the first pair of legs (Fig. 1*a*)..... *Phryganea*
   Coxal combs of the second pair of legs (Fig. 2*b*) smaller and less strongly developed than those of the first legs (Fig. 2*a*), not projecting from the surface of the coxa as prominently as above..... *Agrypnia*

3. Mesonotum with two prominent brownish-yellow sclerites on the anteromesal surface (Fig. 15)..... *Oligostomis*
   Mesonotum with the mesal area entirely membranous and without sclerites, except for the bases of the mesal setae, which sometimes have a small sclerotized ring (Figs. 13, 14).... 4

4. Anterior edge of the pronotum entirely margined with a dark-brown band, the central area behind this band mainly uniform yellow (Fig. 10)..... 2
   Anterior edge of the pronotum mostly yellow, the central area traversed by a pair of dark-brown bands either longitudinally (Fig. 12), obliquely (Fig. 11), or transversely (Fig. 14)..... 5

5. Mesonotum and metanotum with two irregular, longitudinal dark bands (Fig. 13); larval case built as a single spiral band (Fig. 6)..... 6
   Mesonotum and metanotum without these dark bands, with several narrow unpigmented bands, or fairly uniform in pigmentation (Fig. 14); larval case built as a series of separate rings joined together (Fig. 5)..... 7

6. Throughout a large part of North America, although not recorded from Alaska.. *Banksiola*
   Confined, in North America, to western Alaska..... *Oligotricha*

7. Frontoclypeus without dark-brown bands, although the lateral edges with diffuse brown patches (Fig. 14)..... *Ptilostomis*
   Frontoclypeus with two dark-brown bands..... *Hagenella*?

[2]Larvae of the genus *Fabria*, with two species, *inornata* (Banks) and *complicata* (Banks), have not yet been discovered.

### *Phryganea* Linnaeus

Only two North American species are known in this genus, *cinerea* Walker and *sayi* Milne. Larvae have been associated with both but distinguishing structural characters which would permit their separation have not yet been found. The distribution of the two species is, however, one aid to the separation of their larvae: *cinerea* is more northern and is transcontinental, extending from Alaska to Newfoundland and Massachusetts, and into the western montane areas of the United States; *sayi* is restricted to the eastern central region, extending from Maine to North Carolina and west to Missouri and southern Wisconsin. The two species overlap in a broad belt extending from southern New Hampshire through southwestern Ontario, southern Michigan, to southern Wisconsin.

The following description of the larva (Fig. 16, *cinerea*) thus applies to both species. Head yellow, the dorsal bands fairly wide; frontoclypeus with a median dark-brown band; ventral surface of the head without prominent markings, the gula and bordering edges of the genae brown. Pronotum yellow, the anterior edge completely bordered with a heavy black line, followed by a dark-brown band of variable width, which is frequently extended some distance posteromesad; posterior portion of the pronotum with variable brown spots; mesonotum and metanotum of preserved specimens purplish in color, each with two narrow, unpigmented, longitudinal bands intersected by a transverse, arcuate, unpigmented band; behind this arcuate band and between the two longitudinal bands is a pair of short, unpigmented, curved bands. Coxal combs of the first and second pairs of legs very strongly developed (Fig. 1), projecting strongly from the coxa in profile, the patch of combs on the first legs more extensive than that on the second; coxal combs of the third legs reduced to minute points visible only under high magnification. Abdominal gills complete on the first seven segments; the eighth segment with anterior gills only, and these varying from one to three in each species; dorsal plate of the ninth segment rather wide and short, yellow or lightly marked with brown; lateral sclerites of the anal legs yellow, the basal area lightly marked with brown. Length of full grown larva about 40 mm.

Larvae of *Phryganea* live in lakes, small ponds, and rivers. I have not found them in small, cool streams of the type inhabited by larvae of *Oligostomis*. The larval case is constructed as a spiral (Fig. 6) which may be either dextral or sinistral. The larvae spin a very stout anterior sieve membrane before pupating. The pupal mandibles are well developed and heavily sclerotized with a stout cutting blade.

The larva and pupa of *sayi* (= *interrupta* Say) have been described by Vorhies (13), and by Lloyd (3), while Neave (5) has described the larva and pupa of *cinerea*. Neave has noted variation in the markings of the larva of *cinerea*, although his difficulty in comparing his own material with Lloyd's (3) description of *cinerea* arises from the fact that Lloyd did not actually have *cinerea* at all. Lloyd's specimens were probably *Banksiola smithi*. Ross (7) has also described and figured larval and pupal structures for *Phryganea*. Milne's

(4) reference to larvae of *sayi* in Lake Manitoba places this species beyond the range known for it, and I assume that this reference really pertains to *cinerea*.

## *Agrypnia* Curtis

Of the nine North American species assigned to this genus, larvae were previously known for three, *straminea*, *vestita*, and *pagetana*, while the larva of a fourth, *improba*, is described here. Most of the five remaining species for which larvae have not yet been discovered occur across Canada and in some of the montane areas of the western United States.

All larvae now known in this genus construct cases of the spiral type (Fig. 6). Larvae of at least two species, *vestita* and *improba*, do not spin an anterior sieve membrane prior to pupation. The pupal mandibles are fully sclerotized and have a well-developed apical blade. The larvae live in ponds, lakes, and slow-flowing rivers and streams.

### Key to the Known Larvae of North American Species of *Agrypnia*

1. Pronotum with a pair of oblique, dark-brown bands, extending from the anterolateral corners, converging posteriorly, and coming very close together near the posteromesal margin (Figs. 7, 8)..... 2
   Pronotum without these oblique, dark-brown bands; the anterior margin completely or partially edged with dark brown, the central area mostly uniform yellow and without markings (Fig. 10), or with a few small brown spots (Fig. 9)..... 3

2. Eighth abdominal segment with the anterolateral pair of gills present.....*straminea*
   Eighth abdominal segment with no gills.....*improba*

3. Dorsal bands of the head broad; frontoclypeus with a median, dark-brown band extending over its full length (Fig. 9).....*pagetana*
   Dorsal bands of the head not as broad; frontoclypeus without dark-brown markings, or sometimes with a short brownish area (Fig. 10).....*vestita*

*Agrypnia improba* (Hagen)

*Larva* (Fig. 7).—Similar to *straminea*, differing mainly in the absence of certain of the abdominal gills, in the more irregular band of the frontoclypeus, and in the larger size of the full grown larva. Head yellow, dorsal bands broad; frontoclypeus with a narrow, rather irregular, median, light-brown band, not quite reaching either end of the frontoclypeus; ventral surface of the head with a broad, irregular brown patch extending from the anterior edge of each gena, almost to the posterior edge. Pronotum yellow, with a pair of oblique, dark-brown bands extending from the anterolateral corners, converging posteriorly, and almost joining near the posteromesal margin; mesonotum and metanotum generally dark in color, the dark portion broken into several separate areas by broad, unpigmented bands. Coxal combs of the first pair of legs fairly large and well developed in a broad band along the posterior edge of the coxa, smaller and scattered on each side of this band; coxal combs of the second pair of legs scattered and for the most part smaller, but again, the larger combs along the posterior edge of the coxa, the smaller ones at each side of these; coxal combs of the third pair of legs very small, appearing as tiny raised points unless highly magnified. Abdominal gills complete on the

first five segments; sixth segment with the anterodorsal gills absent, the anterolateral gills variable; seventh segment with the anterolateral gills absent, the anterodorsal and posterodorsal gills variable; eighth segment without gills; dorsal plate of the ninth segment yellow with a brown patch covering most of the anterior two-thirds of the plate; lateral sclerites of the anal legs yellow with a brown dorsobasal patch. Length of full grown larva about 30 mm.

This species is widespread in northern North America, occurring from British Columbia to Newfoundland and south to North Carolina.

*Agrypnia straminea* Hagen

*Larva* (Fig. 8).—Similar to *improba*, differing mainly in the presence of certain of the abdominal gills, in the more regular band on the frontoclypeus, and in the smaller size of the full grown larva. Head yellow, dorsal bands of moderate width; frontoclypeus with a median dark-brown band of moderate width, the band fairly regular throughout and extending from the anterior margin of the frontoclypeus almost to the posterior point; ventral surface of the head with a brown patch that is somewhat smaller and lighter than in *improba*. Pronotum, mesonotum, and metanotum as in *improba*. Coxal combs of all three pairs of legs as in *improba*. Abdominal gills complete on the first seven segments, but only the anterolateral gills present on the eighth segment; dorsal plate of the ninth segment and lateral sclerites of the anal legs as in *improba*. Length of full grown larva about 20 mm.

This species is widespread in northern North America, occurring from the Northwest Territories to Newfoundland, south to northern Illinois.

The larva and pupa of *straminea* have been described by Elkins (1). The absence of so many of the abdominal gills in his description of the larva has probably resulted from the use of larvae which were not fully grown. The larva figured by Ross (7) as *Banksiola selina* (= *crotchi*) probably belongs to *Agrypnia straminea*.

*Agrypnia vestita* (Walker)

*Larva* (Fig. 10).—Not similar to any of the other known North American species. Head yellow, dorsal bands fairly broad; frontoclypeus usually without brown markings, but in some specimens there is a short diffuse brownish patch; ventral surface of the head with a diffuse brownish area on the mesal portion of each gena, along each side of the gula. Pronotum yellow, the whole anterior edge bordered with a dark-brown band, the central area without prominent markings, but sometimes with a few brownish spots near the posterior margin; mesonotum and metanotum with broad unpigmented bands separating pigmented areas as in *improba*. Coxal combs of the first pair of legs (Fig. 3) smaller than in the other species of *Agrypnia*, the largest combs along the posteroventral edge, smaller combs on each side; coxal combs of the second pair of legs more uniform, about the same size as the smaller combs on the first legs; coxal combs of the third pair of legs very small as in *improba*. Abdominal gills complete on the first four segments; fifth segment with the

posterodorsal gills absent; sixth segment with the anterodorsal and posterodorsal gills absent; seventh segment with the anterodorsal, posterodorsal, and posteroventral gills absent; eighth segment without gills; dorsal plate of the ninth segment and lateral sclerites of the anal legs as in *improba*. Length of full-grown larva about 25 mm.

The species is restricted to the eastern half of North America, extending from North Dakota to Maine, south to Alabama and South Carolina.

Larval and pupal structures of this species have been figured by Ross (7). The larvae described as *vestita* by Lloyd (3) are not of this species, but probably belong to *Banksiola crotchi*.

*Agrypnia pagetana* Curtis

My material of this species is restricted to a single mature larva from Germany, some larvae from the Northwest Territories that almost certainly belong to this species, and larval sclerites with the pupal skin from an adult reared at Churchill, Manitoba. This, together with the description by Silfvenius (9), forms the basis of the following description. The arrangement and variations of the abdominal gills are given by Silfvenius.

*Larva* (Fig. 9).—Not similar to any of the other known species. Head brownish yellow, dorsal bands broad; frontoclypeus with a broad, median, dark-brown band extending over its full length; these dark bands are wide and the light ground color of the head is reduced to narrow strips between them; ventral surface of the head with the genae fairly uniform light brown, a few dark-brown spots posteriorly, and the gula lighter. Pronotum brownish yellow, the anterior margin and the adjoining third of the pronotum dark brown, the central mesal area with irregular dark-brown spots, the lateral areas lighter and without spots; mesonotum and metanotum not as in *improba*, but more uniformly light purplish, the pigmented area broken only by slender unpigmented bands, very much as in *Phryganea* in size and arrangement. Coxal combs of the first pair of legs fairly well developed and prominent, with the patch of larger combs on the posteroventral edge somewhat more extensive than in *improba*; coxal combs of the second pair of legs smaller and more uniform; coxal combs of the third pair of legs very small as in *improba*. Dorsal plate of the ninth segment and lateral sclerites of the anal legs as in *improba*. Length of full grown larva about 20 mm. Although larvae of *pagetana* usually construct a spiral case typical for this genus, they occasionally make use of a hollow plant stem as a case.

This species occurs throughout the northern portions of North America, Europe, and Asia.

## *Banksiola* Martynov

The five species known in this genus are confined to North America, with larvae for three of these described here. Still unknown are the larvae and pupae of two eastern species, *calva* Banks and *concatenata* (Walker). More complete information on the distribution of these species will be found in a

recent revision of the genus (Wiggins (14)). Larvae of the three species known construct cases of the spiral type (Fig. 6), and they do not spin an anterior sieve membrane prior to pupation. The pupal mandibles are reduced to short semimembranous lobes.

## KEY TO THE KNOWN LARVAE OF *Banksiola*

1. Pronotum with two longitudinal, parallel, dark-brown bands (Fig. 12).....*dossuaria*
   Pronotum with two oblique, dark-brown bands, extending from the anterolateral corners and converging toward the posteromesal margin (Fig. 13)..... 2
2. Frontoclypeus with a median, dark-brown band (Fig. 11).....*smithi*
   Frontoclypeus without dark-brown markings (Fig. 13).....*crotchi*

*Banksiola crotchi* Banks

*Larva* (Fig. 13).—Not similar to any other species known. Head yellow, dorsal bands broad; frontoclypeus without brown markings; ventral surface of the head without central dark patches, the anterior and posterior edges of the genae somewhat brownish. Pronotum yellow, with a pair of broad, oblique, dark-brown bands extending from the anterolateral corners, converging posteriorly, the ends sometimes joining near the posteromesal margin; mesonotum and metanotum light in color, each with a pair of dark, irregular, subparallel bands. Coxal combs present on all three pairs of legs, but they are exceedingly small, and appear as tiny raised points (Fig. 4). Abdominal gills of the first six segments complete; seventh segment with the posteroventral gills absent; eighth segment with only the anterodorsal gills present; dorsal plate of the ninth segment rather long in relation to its width, fairly uniform brown; lateral sclerities of the anal legs yellow, with the posterodorsal edge brown. Length of full-grown larva about 20 mm.

Larvae of this species live in a wide variety of aquatic habitats including lakes, small ponds, slow-flowing rivers, and sluggish, rather warm streams.

This is the only transcontinental species in the genus. It has not, however, been recorded from Alaska, to which *Oligotricha lapponica*, the only North American species of the somewhat similar genus *Oligotricha*, is restricted.

It has already been pointed out (Wiggins (14)) that the larva associated with *B. crotchi* (= *selina* Betten) by Ross (7) does not belong to that species. The markings shown on the mesonotum of the specimen in Ross's Fig. 562 are more like those of *Agrypnia*, rather than of *Banksiola*, and the figure probably represents *Agrypnia straminea*. On the other hand, the larva described as phryganeid genus A by Ross (7, Fig. 566) is identical with the larva of *Banksiola crotchi*, even to the dark bands on the mesonotum. I believe that this larva, genus A, is actually *B. crotchi*. These same corrections would also apply to the keys to the larvae of phryganeid genera given by Pennak (6) and Ross (8) which are based on the 1944 key. Lloyd (3) described a larva which he called *Phryganea vestita* (= *Agrypnia vestita*). His larvae do not belong to that species, and there is every appearance that these are really larvae of *Banksiola crotchi*.

*Banksiola smithi* (Banks)

*Larva* (Fig. 11).—Not similar to any other species known. Head yellow, dorsal bands rather narrow; frontoclypeus with a median dark-brown band; ventral surface of the head with an irregular brownish line extending from the angle on the anterior edge of each gena, and almost reaching the posterior edge. Pronotum yellow, with a pair of oblique, dark-brown bands extending from the anterolateral corners, converging posteriorly, and almost meeting near the posteromesal edge of the pronotum; mesonotum and metanotum each with a pair of dark longitudinal bands as in *crotchi*. Coxal combs as in *crotchi*. Abdominal gills of the first six segments complete, but the seventh segment with the posteroventral gills absent, and the anterodorsal gills variable; eighth segment without gills; dorsal plate of the ninth segment and lateral sclerites of the anal legs as in *crotchi*. Length of full grown larva 15 to 20 mm.

Larvae have been collected in ponds and sluggish streams.

This species was previously known from the northeastern United States, and has recently been collected in Ontario and Quebec.

Lloyd's (3) description of a larva which he called *Phryganea cinerea* is not that species, but of all the phryganeid larvae now known, it appears to be closest to *Banksiola smithi*, even to the absence of gills on the eighth abdominal segment. Lloyd's larvae were taken at Old Forge, New York, where adults of *smithi* have also been taken.

*Banksiola dossuaria* (Say)

*Larva* (Fig. 12).—Not similar to any other species known. Head yellow, dorsal bands broad; frontoclypeus with a somewhat lighter and narrower median brown band; lateral bands shorter, wider, and more irregular than in either *crotchi* or *smithi;* ventral surface of the head with an irregular brownish patch on the posterior half of each gena. Pronotum yellow, with a pair of longitudinal, parallel, broad, dark-brown bands; mesonotum and metanotum each with a pair of dark longitudinal bands as in *crotchi*. Coxal combs as in *crotchi*. Abdominal gills, dorsal plate of the ninth segment and lateral sclerites of the anal legs as in *crotchi*. Length of full grown larva about 20 mm.

Larvae have been collected in a pond and in a sluggish stream.

This species occurs in southeastern Canada and in the eastern United States.

## *Oligostomis* Kolenati

The larvae of the two North American species, *ocelligera* (Walker) and *pardalis* (Walker),[3] recognized in this genus are known, but reliable characters for their separation have still not been found. Relative size of the full grown larvae remains the only guide. Mature larvae of *pardalis* reach a length of about 30 mm, while mature larvae of *ocelligera* reach a length of about 20 mm. Both species are confined to eastern Canada and the northeastern United States, where they inhabit small cool streams, usually with an appreciable

[3]Reasons for assigning *pardalis* to *Oligostomis* rather than to *Eubasilissa*, as has been done by other authors, have been given previously (Wiggins (15)).

current. The larvae construct cases of the ring type (Fig. 5). The anterior sieve membrane is not spun by *pardalis*, although it is not yet known what the behavior of *ocelligera* is in this regard. The pupal mandibles are represented by semimembranous lobes.

The following description of the larva (Fig. 15, *pardalis*) thus covers both species. Head yellow, dorsal bands of moderate width; frontoclypeus with a narrow, irregular dark-brown patch along each side, separated from the adjacent dorsal band on each side only by the frontoclypeal suture; ventral surface of the head with a broad, somewhat elliptical dark-brown patch on the central area of each gena. Pronotum yellow, the anterior portion without brown markings, the central and posterior portion on each side with a somewhat angulate dark-brown patch; mesonotum and metanotum without prominent markings, although the same arrangement of narrow unpigmented bands as in many of the other genera such as *Phryganea* and *Ptilostomis* is very faintly represented; mesonotum with a pair of prominent, anteromesal, yellowish sclerites, each one with a central brownish patch. Coxal combs of all three pairs of legs very small, appearing under moderate magnification as tiny raised points. Abdominal gills of the first four segments complete; the anterodorsal gills absent from the fifth, sixth, and seventh segments; eighth segment without gills; dorsal plate of the ninth segment fairly long in relation to its width, yellow, the anterior half with scattered dark-brown spots; lateral sclerites of the anal legs yellow with a few brownish spots.

Larvae of both species and the pupa of *pardalis* have been described by Lloyd (3), although *ocelligera* is referred to by the synonym *stygipes*.

### *Oligotricha* Rambur

Only one species, *lapponica* (Hagen), is known in North America, where it is restricted to western Alaska. This same species also occurs in northern Europe and Asia, and the larva and pupa were described by Silfvenius (11). I have been unable to obtain larval material of this species. As far as is known, the larval case in this genus is of spiral construction and the pupal mandibles are fully sclerotized and well developed with an elongate cutting blade.

### *Ptilostomis* Kolenati

All four species assigned to this genus are confined to North America. Two of these, *ocellifera* (Walker) and *semifasciata* (Say), are transcontinental, while the other two, *postica* (Walker) and *angustipennis* (Hagen), are restricted to the east. I have reared both *ocellifera* and *semifasciata*, but have not found reliable characters to distinguish the larvae. The larvae and pupae of *postica* and *angustipennis* are unknown.

The larvae of both species reared construct cases of the ring type (Fig. 5), but do not spin an anterior sieve membrane before pupating. The pupal mandibles in both species are reduced to short semimembranous lobes. Larvae of *ocellifera* live in cool waters, often in the same streams as species of *Oligostomis*, or in cool spring pools. Larvae definitely established as *semifasciata*

have only been taken once, in a drainage canal on Walpole Island, Lake St. Clair, Ontario, a habitat that seems somewhat warmer than those from which larvae of *ocellifera* have been reared.

The following description of the larva (Fig. 14, *ocellifera*) applies to both *ocellifera* and *semifasciata*. Head yellow, dorsal bands fairly wide; frontoclypeus with a few small and irregular dark-brown patches along each side, similar to the lateral patches in *Oligostomis*, but not as extensive; ventral surface of the head uniform yellow, without markings, the gula brownish. Pronotum mainly yellow, but with an irregular, dark-brown, arcuate band extending across the meson; mesonotum and metanotum with narrow unpigmented bands as in *Phryganea*. Coxal combs on all three pairs of legs minute, appearing under moderate magnification as tiny raised points. Abdominal gills of the first seven segments complete; eighth segment with only the anterolateral and anteroventral gills present; dorsal plate of the ninth segment fairly deep as in *Oligostomis*, yellow, the anterior half with scattered dark-brown spots; lateral sclerites of the anal legs yellow with a basal brownish patch. Length of fully grown larva about 25 mm.

The larva and pupa of *ocellifera* have been described by Vorhies (13) under the name *postica*, and also by Ross (7). Lloyd (3) described the larva of a species of *Ptilostomis* which he identified as *postica*, but this could well be *ocellifera* since the two were often confused as adults at that time.

### *Hagenella* Martynov

There is only one North American species, *canadensis* (Banks), and it is restricted to eastern Canada and the northeastern United States. The larva and pupa of this species have not yet been discovered, and in the absence of any other evidence, the position of the genus in the preceding key is based on characters described for the European species *H. clathrata* (Kol.), the one species of five in the genus for which the larva and pupa are known. In *clathrata* the larval case is reported to be of the ring type, and the pupal mandibles are reduced to short, semimembranous lobes (Silfvenius (10); Struck (12)).

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University, kindly provided reared material of *Agrypnia pagetana* from his own work at Churchill, Man. Other larvae were made available through the Entomology Research Institute from the Canadian National Insect Collection, Canada Department of Agriculture, Ottawa. From Dr. Walter Döhler of Klingenberg, Germany, I have been able to obtain larvae of some of the European Phryganeidae.

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# HYBRIDIZATION EXPERIMENTS IN RHODEINE FISHES (CYPRINIDAE, TELEOSTEI)

## THE INTERGENERIC HYBRIDS OF ACHEILOGNATHUS LANCEOLATA ♀ × ACANTHORHODEUS ATREMIUS ♂ AND TANAKIA TANAGO ♀ × ACHEILOGNATHUS RHOMBEA ♂[1]

J. J. Duyvené de Wit


## Abstract

In order to study the phylogenetic relations between rhodeine species a number of interspecific and intergeneric crossings have been performed. This communication deals with the intergeneric hybrids obtained from the combinations *Acheilognathus lanceolata* ♀ × *Acanthorhodeus atremius* ♂ and *Tanakia tanago* ♀ × *Acheilognathus rhombea* ♂.

From the first combination adult hybrids have been obtained which were intermediate between both parental species and showed a male phenotype. The second combination produced larvae, but a deficient yolk supply prevented them from reaching the free-swimming stage.

Experimental evidence is presented for the occurrence of a dominant genetic factor in *A. rhombea* males, which controls the duration of embryonic development in this and other bitterling species.


## Introduction

A number of hybridization experiments in rhodeine fishes have been performed under laboratory conditions with a view to investigating the presence and the degree of genetic affinity possibly existing between representative species belonging to the Rhodeinae.

The group ranks as a subfamily and is represented in Taiwan, Japan, Korea, China, Russia, and Western Europe. Unlike most other fishes, bitterlings are unable to propagate without the aid of fresh-water mussels which act as incubators of their eggs.[2] In a previous publication the peculiar mode of propagation of the Western European bitterling has been described by the present author (1).

Some kinds of hybrids, which have been produced in our laboratory, appeared to represent excellent experimental objects for studying the genetics of separate elements of spawning behavior which are considerably different in the parental species from which the hybrids were produced.[3]

*Acheilognathus lanceolata* (Temminck & Schlegel) and *Acanthorhodeus atremius* (Jordan & Thompson) are incapable of interbreeding because of incompatibility of their spawning behavior. In the combination *Tanakia tanago* (Tanaka) and *Acheilognathus rhombea* (Temminck & Schlegel) the difference in body size (see Fig. 2) is too great to allow successful interbreeding, although the behavior patterns of both species are fairly similar. Therefore, artificial insemination has been used in the present experiments.

[1]Manuscript received June 27, 1960.
Contribution from the Department of Zoology, University of the Orange Free State, Union of South Africa.

[2]*Sarcocheilichthys* spp. also spawn their eggs into bivalves by means of an elongated urogenital papilla (ovipositor), thus representing a remarkable case of convergency.

[3]The experiments dealing with the ethology of bitterling species and their hybrids will be published at a later date.

## Materials and Methods

The bitterlings used in our experiments were imported from Japan and kept in well-aerated aquaria at room temperature. They were fed deep-frozen fish roe and a commercial dry compound preparation which meets the requirements of omnivorous pet fishes.

In order to stimulate breeding activity each aquarium was provided with three bivalves (*Aspatharia wahlbergi*, Krauss and/or *Unio caffer*, Krauss).

Artificial insemination was performed in the usual way and after fertilization the eggs were placed in covered Petri dishes partly filled with tap water. Incubation took place at room temperature and the cultures were thoroughly cleaned twice a day.

## Results and Discussion

*The Interspecific Hybrids of A. lanceolata* ♀ *and A. atremius* ♂

Forty-eight eggs of one *A. lanceolata* female were inseminated with sperm of one *A. atremius* male. In 47 eggs fertilization was complete. After hatching and rearing, 42 larvae reached the free-swimming stage. Fourteen hybrids were arbitrarily selected and have been raised to the adult stage. A representative adult hybrid specimen is illustrated in Fig. 1, together with specimens of the parental species.

All hybrids showed a male phenotype. They were uniform in size and intermediate between the parental species. In the spawning season, all hybrids displayed full breeding colors but sexual behavior, as tested against mature *Rhodeus ocellatus* (Kner) females, was weak. Tubercles, indicating hormonal gonadal activity, were present on the top of the snout. However, spermatogenesis and sperm motility were much impaired.

The taxonomic description of the present hybrid form will be published separately.

*The Intergeneric Hybrids of T. tanago* ♀ *and A. rhombea* ♂

Twenty-four eggs of two *T. tanago* females were inseminated with sperm of one *A. rhombea* male. Fertilization was complete and all eggs hatched within 40 hours. Because the yolk volume in the eggs of *T. tanago* is about half of that in the eggs of *A. rhombea*, the larvae did not reach the free-swimming stage due to starvation. A representative specimen of a 31-day-old larva is illustrated in Fig. 2, together with adult specimens of the parental species.

*Genetic Control of Duration of Embryonic Development*

In their natural habitats the breeding season of *T. tanago* is in the spring and that of *A. rhombea* in the autumn. The development in *T. tanago* from fertilization to the attainment of the free-swimming stage takes 18 days, whereas in *A. rhombea* the process continues over 4–5 months. When incubated at room temperature the period of embryonic development in this species remains practically unchanged. Therefore, the presence of a genetic determinant for delaying embryonic development must be assumed; this

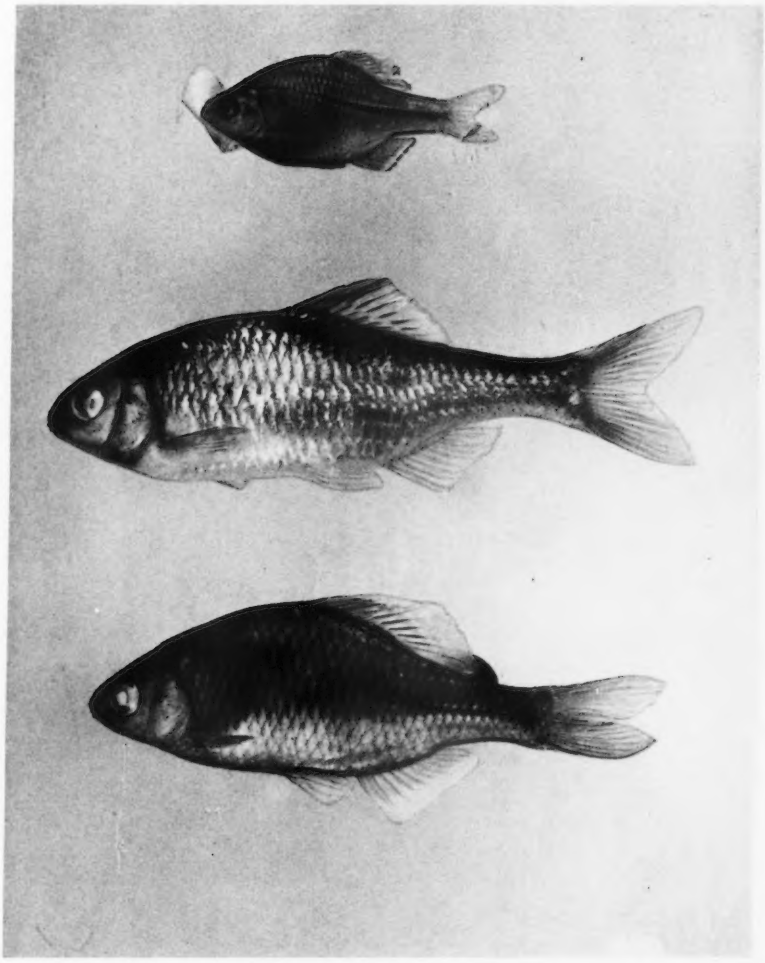

FIG. 1. Top: specimen of a nearly full-grown sexually mature *Acanthorhodeus atremius* male. Standard length 33 mm.
Middle: specimen of a nearly full-grown *Acheilognathus lanceolata* female. The fish is out of season and, therefore, the ovipositor is absent. Standard length 82 mm.
Bottom: specimen of an adult intergeneric hybrid obtained from the combination *A. lanceolata* ♀ and *A. atremius* ♂. Standard length 70 mm.

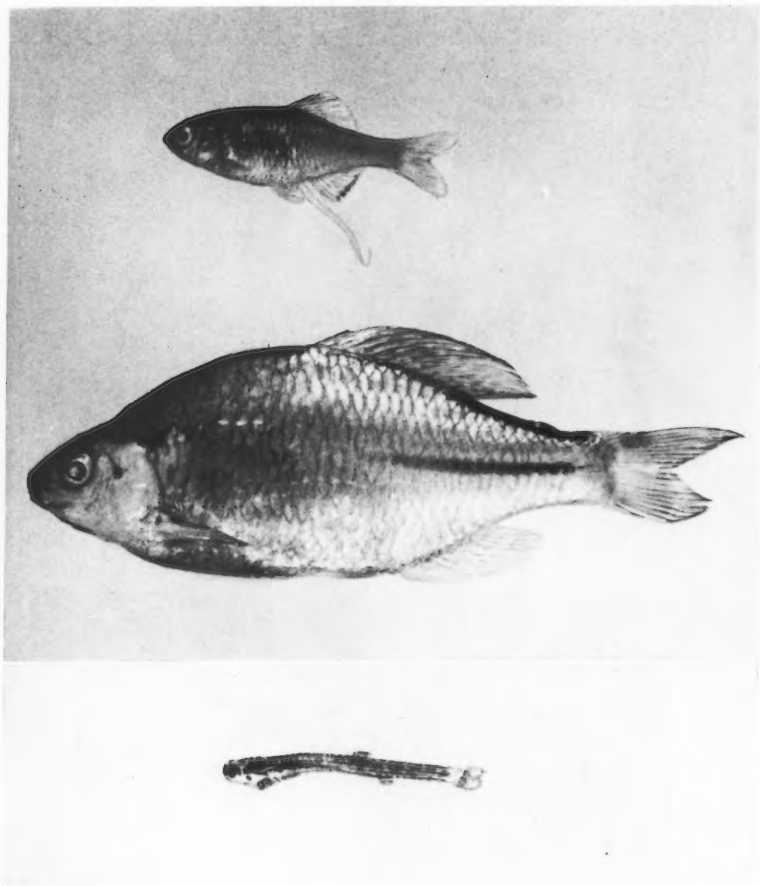

FIG. 2. Top: specimen of an adult *Tanakia tanago* female. Note the presence of the ovipositor in front of the anal fin. Standard length 37 mm.
Middle: specimen of an adult *Acheilognathus rhombea* male. Standard length 88 mm.
Bottom: specimen of a 31-day-old larval intergeneric hybrid obtained from the combination *T. tanago* ♀ and *A. rhombea* ♂. Standard length 6 mm.

determinant acts as a very effective means in preventing unseasonable release of fry by najads accommodating the developing *A. rhombea* larvae.

In the hybrids obtained from *T. tanago* ♀ and *A. rhombea* ♂, embryonic development was still incomplete after 31 days, but it may be assumed that it would have been completed within 36 days, thus extending over a period twice as long as in *T. tanago*. Therefore, the action of a presupposed genetic determinant for developmental retardation in the male genome of *A. rhombea* appears to affect the duration of embryonic development in the hybrid combination with *T. tanago* as well.

The absence of a delaying factor in the genome and cytoplasm of *A. rhombea* females could be demonstrated by inseminating eggs of this species with sperm of *R. ocellatus*. In the latter species, the duration of embryonic development is 18–20 days, whereas in the hybrids of *A. rhombea* ♀ × *R. ocellatus* ♂ it was 25 days. These hybrids reached the free-swimming stage while nearly 70% of their yolk supply had not been utilized. The inhibiting action of yolk on embryonic differentiation may account for the slight prolongation of the embryonic development in these hybrids.

## Acknowledgments

The author is greatly indebted to Professor Tokiharu Abe, Dr. Yoshitsugu Hirosaki, and Mr. Shigeo Kobayashi for supplying the Japanese rhodeine species, and to Mr. F. G. du Jardin for providing photographs of the illustrated specimens. The financial assistance of the Council for Scientific and Industrial Research of the Union of South Africa is gratefully acknowledged.

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# COMPARATIVE STUDY OF DITYLENCHUS DESTRUCTOR THORNE, 1945 (NEMATODA:TYLENCHIDAE), FROM POTATO, BULBOUS IRIS, AND DAHLIA, WITH A DISCUSSION OF DE MAN'S RATIOS[1]

LIANG-YU WU

## Abstract

Nematodes having the general characters of *Ditylenchus destructor* Thorne, 1945, from the potato, bulbous iris, and dahlia, did not reveal any significant difference in the basic structures to suggest the presence of more than one species. However, due to host influence, there may be some variation in total length, width, number and arrangement of sex cells, length of oesophagus, length of spear, and tail shape. Nematodes from potato were successfully transferred to dahlia and carrot, and from iris and dahlia to potato.

Specimens of *D. destructor* from potato interbreed freely with specimens from bulbous iris and from dahlia. Evidently these nematodes from the three hosts are the same species, i.e., *D. destructor*.

Among the ratios used, the values of *c* in the case of males and that of "*V*%" have some support from the data.

## Introduction

Because of lack of information on the range of characters of *Ditylenchus destructor* Thorne, 1945, there has been some uncertainty about the identity of nematodes having the general characters of this species that have been found on host plants other than the potato. As a first step in attempting to resolve this problem a morphological study of *D. destructor* was made (5). This has now been followed by comparative studies of nematodes from potato, bulbous iris, and dahlia.

## Materials and Methods

The infested bulbous iris and dahlia were obtained from British Columbia, Canada, and from Portland, Oregon, U.S.A., respectively.

Nematodes were fixed in formalin–triethanolamine solution, stained with cotton blue, and mounted in lactophenol. Morphological studies were made chiefly with living specimens, but specimens mounted in lactophenol were also used.

Pieces of infested iris bulbs and dahlia tubers were put into separate boxes and pots with uninfested potato plants in the greenhouse. A few months later new potatoes were found infested with *Ditylenchus* sp. The nematodes found in these newly infested potatoes were studied.

*D. destructor* of potato origin also experimentally infested healthy carrots and dahlia in the greenhouse bed and the nematodes subsequently recovered were studied.

[1]Manuscript received July 18, 1960.
Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

Potatoes used in these experiments were from the Central Experimental Farm, Department of Agriculture, Ottawa. All tubers were washed and dried. A long incision, very close to the surface of the potato, was made with a sterilized scalpel. Previous tests had shown that the nematodes did not live well in healthy tissue. Therefore a red-hot dissecting needle was inserted into the incision. Thus a strip of tissue in the incision was burnt. Consequently, the number of positive growths in the potato incisions was fairly high. It seemed that this strip of burnt tissue had improved the chances of the nematodes becoming established.

Male and female specimens were selected. They were thoroughly washed with sterile, cold water. One pair or more were inoculated into the incision with a fine pipette. The opening was sealed with petroleum jelly or wax. The potatoes were kept in the laboratory at room temperature, in jars or plastic bags, to prevent excessive evaporation.

## Results

*A. Morphology*

The basic parts in the various organ systems were found to be similar to those described for *D. destructor* (5), but some minor differences were noticed. These were as follows:

*1. Nematodes Found in Bulbous Iris*

Ten males and 18 females examined.

*Reproductive system.*—Spermatogonia in testis usually in a single row. A few pairs sometimes present near the proximal end or at irregular intervals in the testis. Sex cells in the vas deferens and in the seminal vesicle less numerous. In the ovary, oöcytes usually in a single row; a few pairs occasionally present near the proximal end. Tip of ovary, in a few cases, reflexed. Cuticular lining of vagina and lips of vulva fairly thick. Lining of vagina appears to be more conspicuous than that in the potato form.

*Tail.*—Caudal end of tail of female appears to be more nearly cylindrical (Fig. 1, F) than that from potato (Fig. 1, A).

*Spears.*—Slightly longer in both males and females than those from potato (Tables I, II).

*Oesophagus.*—Proportionately longer, despite shorter body length.

*Oesophageal gland.*—Dorsal gland appears to be smaller and shorter, in some cases extending only slightly backward over anterior end of intestine. Glands very clear and not so granulated.

*2. Nematodes from Iris Grown in Potato*

Twenty-three males and 25 females examined.

*Reproductive system.*—Number of sex cells greatly increased. In the testis the number ranged from 19 to 58, usually in single or double rows at irregular

intervals, but in some cases in triple rows. In the vas deferens the number of spermatocytes ranged from 26 to about 90, mostly in double rows, and in some cases in multiple rows.

In the ovary the number of oöcytes ranged from 19 to 59. Cells at both ends of the ovary in single rows, those in the middle region usually in double rows, the number varying from a few pairs to as many as 19 pairs.

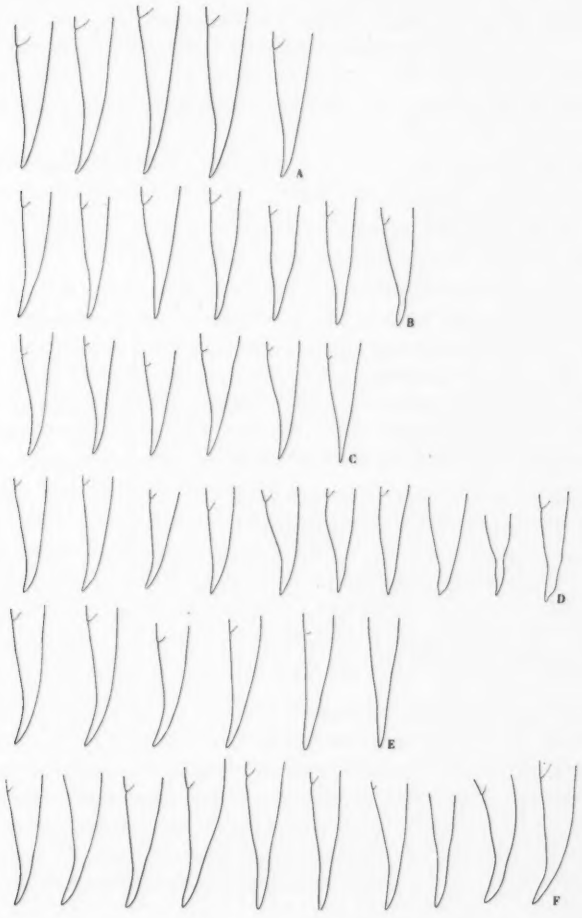


FIG. 1. Tails of *Ditylenchus destructor* from various host plants: A, potato origin; B, potato–dahlia; C, dahlia–potato; D, dahlia origin (last three tails showing irregularities); E, iris–potato; F, iris origin.

*3. Nematodes Found in Dahlia*

Eighteen males and 25 females examined.

*Reproductive system.*—Spermatogonia fewer in number and usually in a single row. The number of spermatocytes in the vas deferens also smaller, only four to eight cells in many cases; consequently, the number of sex cells in the seminal vesicle also very small. On two occasions the testis was reflexed at the tip. Oöcytes usually in a single row and fewer in number. In some cases a few cells were found in a double row near the proximal end. Among 25 specimens examined, one ovary had the tip reflexed. The cuticular lining of the lips of the vulva in some cases seemed thinner and, consequently, the vulva was not prominent.

*Tail.*—The general shape of female tail much like that from potato but some cases with irregularities (Fig. 1, D).

*Spears.*—Slightly shorter in both sexes than those from potato (Tables I, II); apparently the shortest of the three groups studied.

*4. Nematodes from Dahlia Grown in Potato*

Twenty-nine males and 26 females examined.

*Reproductive system.*—Showed a good development of sex cells. The number of spermatogonia varied from 18 to 39. Sixteen cases showed cells in a single row; in others, varying numbers of pairs of cells, from 2 to as many as 14 pairs. In the vas deferens a large number of spermatocytes, from 22 to 54, irregularly in single, double, or multiple rows. The number of oöcytes varied from 13 to 52. Eleven specimens had cells in a single row; others had from 1 to 10 pairs of cells at irregular intervals near the middle of the ovary.

*5. Nematodes from Potato Grown in Dahlia*

Nineteen males and 24 females examined.

*Reproductive system.*—Eleven to 28 spermatogonia, all with sex cells in single rows except 1 with one pair of cells in the row. In the vas deferens the number of spermatocytes varied from 18 to 48. The number of oöcytes varied from 9 to 40; 19 specimens with sex cells in a single row, 4 with one pair in the row. Specimen with 40 oöcytes had six pairs in double rows.

*6. Nematodes from Potato Grown in Carrot*

Thirty males and 12 females examined.

*Reproductive system.*—The number of spermatogonia varied from 11 to 51; most specimens with cells in a single row, 10 with from one to six pairs at the middle region of the testis. Specimen with 51 spermatogonia had cells in a double row. The number of oöcytes varied from 8 to 27; 10 specimens with cells in a single row, 1 with one pair and 1 with seven pairs at the middle region of the ovary.

*B. Measurements of Seven Groups of Nematodes*

The measurements of the seven groups of nematodes mentioned above (that of potato origin, and of potato origin repopulated in carrot and dahlia; that

of bulbous iris origin, and of bulbous iris origin repopulated in potato; that of dahlia origin, and of dahlia origin repopulated in potato) are listed in Tables I and II.

The nematodes used for measurements here were fixed specimens, and the numbers are presented in the tables. The nematodes referred to in Section A were living specimens.

*C. Interbreeding*

Information derived from morphological studies of these nematodes from different host plants did not reveal the presence of any concretely distinct characters in each group. Consequently the writer had to abandon her initial hypothesis and consider the greater possibility of their belonging to the same species.

The fact that nematodes of bulbous iris and dahlia origins grow well in potatoes suggested that conclusive experimental interbreeding could be conducted in potato tubers in the laboratory where the chance of contamination would be most unlikely. Accordingly, a series of interbreeding tests was conducted.

*1. Young Females and Mature Males*

The young females were examined under the microscope and only those apparently not inseminated were used in these tests. In each case the specimens were inoculated into potatoes with the following results:

(*a*) Three females from iris and six males from potato. Examined after 1 month, 220 nematodes were recovered but the total number was greater than this. The size varied from very tiny to large mature nematodes, indicating the presence of more than one generation. Thirty adults were among those examined. There were also many eggs present.

(*b*) One large female from iris and two males from potato. Examined after 1 month, one female, two males, and six small worms were found.

(*c*) Two females from iris and four males from potato. Examined after 5 weeks, numerous nematodes were found, varying in size from very large to very small, all being active.

(*d*) Three females from iris and five males from potato. Examined after 5 weeks, numerous nematodes were found, from large mature to very small ones.

(*e*) Three females from potato and five males from iris. Examined after 5 weeks, although the incision was contaminated with mold, several hundreds of nematodes of various sizes were found.

(*f*) Two females from potato and three males from iris. Examined after 5 weeks, a few dozen nematodes of various sizes were found, ranging from large to very small.

*2. Fourth-stage Larval Females and Mature Males*

In spite of the positive results from these tests it was felt that the evidence might not be conclusive. In examining the females under the microscope prior

to inoculation, it was difficult to be absolutely sure that they were free from insemination. To check this feature another series of tests was conducted, in which females in the fourth larval stage were used. If offspring should be obtained from these juvenile females without a genital opening, the results would be conclusive. The same inoculation procedure was used and the results were as follows:

(*a*) Four larval females from potato and three males from iris. Tuber examined after 5 weeks, about 40 large and medium nematodes were recovered but the total number was greater than this.

(*b*) Five larval females from potato and four males from iris. Tuber examined after 5 weeks, about the same number of nematodes as above were observed. Size varied from large to minute; all were active.

(*c*) Three larval females from potato and three males from iris. Tuber examined after 25 days, numerous nematodes were observed. The size varied from large mature to very small, most being active.

(*d*) Three larval females from potato and one male from iris. Tuber examined after 1 month, about 40 large and numerous small and minute nematodes were observed, the total number being between 200 and 300.

(*e*) Two larval females from potato and two males from iris. Tuber examined after 26 days, 2 mature females, 1 male, and 11 medium and small nematodes were found.

(*f*) Two larval females from potato and two males from iris. Tuber examined after 26 days, 3 large females, 5 large males, and 29 small nematodes were found.

*3. One Pair of Nematodes in Each Incision*

Ten tests were made. Each potato was inoculated with one fourth-stage larval female from potato and one mature male from dahlia. When the tubers were examined 28 days later, the results were as follows:

(*a*) No nematodes found; incision dry.
(*b*) One large female, 10 males, and nine minute nematodes found.
(*c*) One young female nematode found.
(*d*) No nematodes found.
(*e*) Eleven large specimens, about 25 medium and 40 small nematodes found, sizes indicating more than one generation.
(*f*) Very successful growth; numerous nematodes found; sizes varying from large to minute.
(*g*) No nematodes found. Inoculation area spoiled by bacteria.
(*h*) Sixteen large and 30 medium and small nematodes were recovered but the total number was greater than this. Only a few worms were active, the others being dead or inactive.
(*i*) Incision dry. No nematodes found.
(*j*) One dead young female found.

## Discussion

Evidence from the present study indicates that host plants may exert certain influences in producing some minor variations in characters, such as in the size, in the shape of the tail (Fig. 1), in the thickness of the cuticle, in the length of the spear, in the degree of the production of sex cells, and in the length of the oesophagus.

A comparison of nematodes from iris and dahlia with those from potato showed a smaller production of sex cells in both male and female from iris and dahlia. The reproductive systems of both male and female nematodes from iris and dahlia showed better development, with more sex cells, when grown in potato. Similarly, *D. destructor* from potato, grown in carrot and dahlia, also showed a lower production of sex cells in both sexes. Therefore it is felt that the number of sex cells and their arrangement, in single or double rows, etc., should not be stressed too much as a species character. The potato appears to be a very favorable host plant for *D. destructor* as the size is much larger and there is a better production of sex cells than in nematodes grown in the other hosts studied.

From a study of Tables I and II, it will be seen that if any difference between these forms exists, it would seem to be in the length of the oesophagus.

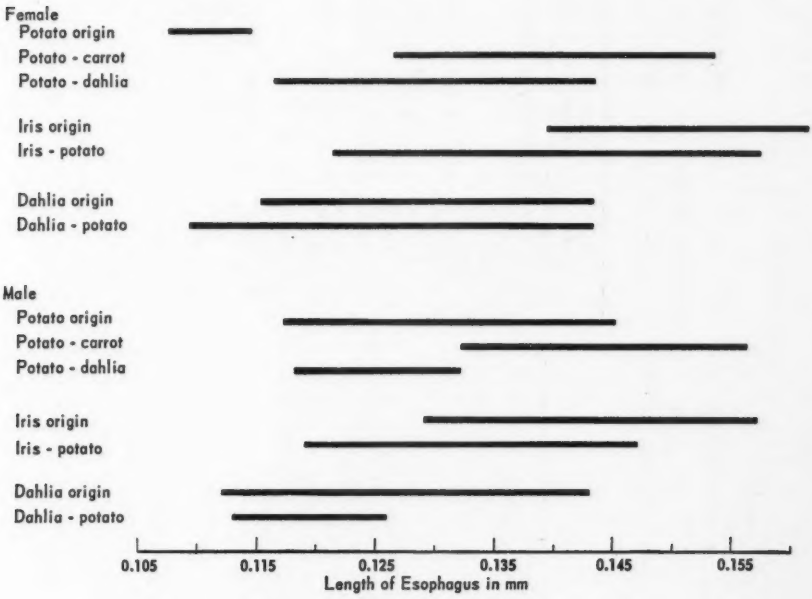


FIG. 2. Length of oesophagus in different host plants.

TABLE I

Measurements of various structures of males from different host plants

| | Host | | | | | | |
|---|---|---|---|---|---|---|---|
| | Potato | Potato–carrot | Potato–dahlia | Iris | Iris–potato | Dahlia | Dahlia–potato |
| Length (mm) | 0.929–1.232 | 0.885–1.115 | 0.741–1.029 | 0.888–1.101 | 0.898–1.054 | 0.625–0.848 | 0.747–0.921 |
| Mean and standard error | 1.072 ± 0.009 | | | 0.964 ± 0.016 | | 0.764 ± 0.017 | |
| Width (mm) | 0.026–0.033 | | | 0.023–0.032 | | 0.018–0.022 | |
| Mean and standard error | 0.030 ± 0.003 | | | 0.026 ± 0.001 | | 0.020 ± 0.004 | |
| Oesophagus (mm) | 0.117–0.145 | 0.132–0.156 | 0.118–0.132 | 0.129–0.157 | 0.119–0.147 | 0.112–0.143 | 0.113–0.126 |
| Mean and standard error | 0.134 ± 0.001 | | | 0.139 ± 0.002 | | 0.125 ± 0.002 | |
| Length of anterior portion (mm) | 0.050–0.062 | 0.052–0.060 | 0.046–0.053 | 0.050–0.060 | 0.046–0.057 | 0.045–0.056 | 0.046–0.052 |
| Length of posterior portion (mm) | 0.069–0.086 | 0.078–0.095 | 0.058–0.081 | 0.077–0.102 | 0.070–0.091 | 0.067–0.087 | 0.065–0.078 |
| Length of posterior portion of oesophagus / Length of anterior portion (mm) | 1.15 –1.58 | 1.37 –1.62 | 1.27 –1.64 | 1.45 –1.85 | 1.40 –1.78 | 1.43 –1.63 | 1.73 –1.68 |
| Length of tail (mm) | 0.062–0.087 | | | 0.063–0.078 | | 0.044–0.059 | |
| Mean and standard error | 0.077 ± 0.001 | | | 0.069 ± 0.001 | | 0.052 ± 0.003 | |
| Distance between anus and anterior end / Length | 92.11–93.88 | 92.22–93.65 | | 91.92–93.74 | 91.89–93.81 | 92.64–94.44 | 92.37–93.71 |
| Length of spear ($\mu$) | 10.2 –11.8 | 10.5 –11.8 | 10.2 –11.0 | 11.1 –12.0 | 10.8 –12.0 | 9.5 –10.9 | 10.0 –11.0 |
| *a* | 32.10–41.29 | 34.06–40.33 | 33.15–44.41 | 28.21–45.04 | 25.71–42.35 | 32.01–42.11 | 28.73–36.52 |
| *b* | 6.30– 9.40 | 7.12– 8.22 | 6.36– 8.29 | 6.16– 8.12 | 6.22– 8.14 | 5.21– 6.81 | 6.02– 7.31 |
| *c* | 12.69–16.29 | 13.43–15.67 | 13.11–16.29 | 12.34–15.97 | 12.33–16.15 | 13.58–16.33 | 13.11–15.90 |
| T | 51.38–73.15 | 57.90–72.83 | | 48.05–67.25 | 59.56–78.21 | 51.12–74.65 | 58.09–76.19 |
| No. of specimens examined | 40 | 15 | 15 | 31 | 18 | 17 | 10 |

TABLE II

Measurements of various structures of females from different host plants

| | Host | | | | | | |
|---|---|---|---|---|---|---|---|
| | Potato | Potato–carrot | Potato–dahlia | Iris | Iris–potato | Dahlia | Dahlia–potato |
| Length (mm) | 0.925–1.396 | 0.923–1.295 | 0.833–1.100 | 0.917–1.291 | 0.994–1.149 | 0.725–1.025 | 0.808–0.992 |
| Mean and standard error | 1.163±0.010 | | | 1.092±0.003 | | 0.863±0.006 | |
| Width (mm) | 0.028–0.043 | | | 0.028–0.042 | | 0.022–0.037 | |
| Mean and standard error | 0.037±0.001 | | | 0.035±0.001 | | 0.029±0.004 | |
| Oesophagus (mm) | 0.107–0.144 | 0.126–0.153 | 0.116–0.143 | 0.139–0.161 | 0.121–0.157 | 0.115–0.143 | 0.109–0.143 |
| Mean and standard error | 0.129±0.001 | | | 0.144±0.004 | | 0.127±0.002 | |
| Length of anterior portion (mm) | 0.049–0.059 | 0.051–0.058 | 0.047–0.054 | 0.053–0.062 | 0.050–0.057 | 0.046–0.055 | 0.045–0.055 |
| Length of posterior portion (mm) | 0.064–0.088 | 0.075–0.098 | 0.068–0.088 | 0.084–0.102 | 0.071–0.098 | 0.069–0.088 | 0.064–0.091 |
| Length of posterior portion / Length of anterior portion (mm) | 1.24 –1.66 | 1.14 –1.76 | 1.33 –1.70 | 1.47 –1.79 | 1.39 –1.67 | 1.20 –1.67 | 1.29 –1.74 |
| Length of tail (mm) | 0.068–0.098 | | | 0.067–0.088 | | 0.050–0.064 | |
| Mean and standard error | 0.083±0.001 | | | 0.075±0.009 | | 0.055±0.002 | |
| Distance between vulva and anterior end (mm) | 0.833–1.156 | | | 0.745–1.068 | | 0.649–0.907 | |
| Mean and standard error | 0.946±0.008 | | | 0.894±0.011 | | 0.751±0.023 | |
| Length of spear ($\mu$) | 10.5 –11.5 | 10.3 –11.5 | 10.6 –11.2 | 11.2 –12.1 | 10.5 –12.0 | 9.5 –10.9 | 10.0 –11.0 |
| *a* | 26.27–39.40 | 24.84–32.01 | 28.32–37.85 | 26.64–37.89 | 29.16–45.59 | 25.22–35.59 | 25.44–31.78 |
| *b* | 7.81–10.60 | 7.21– 9.33 | 6.95– 8.37 | 5.99– 9.09 | 6.72– 9.28 | 5.65– 7.83 | 6.26– 8.03 |
| *c* | 11.67–17.21 | 12.86–15.66 | 13.65–17.22 | 11.33–16.76 | 12.95–16.99 | 13.77–17.67 | 12.83–15.75 |
| *V* | 76.60–83.61 | 80.10–83.53 | 80.75–83.72 | 77.17–83.68 | 77.41–83.00 | 79.69–84.38 | 79.68–82.88 |
| Length of postuterine pouch / Distance between vulva and anus | 54.91–91.22 | 56.91–77.99 | 58.33–81.55 | 40.07–88.97 | 64.27–90.15 | 61.73–93.10 | 73.20–97.83 |
| Distance between vulva and anus / Total length | 9.16–13.90 | 10.11–12.59 | | 9.79–12.76 | 10.16–14.86 | 10.33–12.36 | 10.77–13.87 |
| Distance between vulva and anus / Tail length | 1.30– 2.00 | 1.51– 1.86 | 1.06– 2.00 | 1.38– 2.12 | 1.46– 2.05 | 1.63– 2.12 | 1.38– 2.08 |
| No. of specimens examined | 80 | 15 | 16 | 36 | 21 | 15 | 18 |

The measurements indicate that the forms from bulbous iris and dahlia have a proportionately longer oesophagus, particularly in the form from iris, in spite of a shorter body length. When these two forms develop in potatoes, the length of the oesophagus tends to decrease, shifting back toward that of the form on potato. This tendency is indicated by the graphs (Fig. 2) showing the range of the length of the oesophagus.

Potato nematodes grown in carrot also show a proportionately longer oesophagus. The same result occurs when the potato form develops in dahlia, especially in the case of the female.

The absence of prominent morphological differences in nematodes from these three host plants, coupled with the fact that they interbreed freely, brings the writer to the conclusion that they all belong to the same species.

Steiner (4) reported bulbous iris as a host for *Tylenchus dipsaci*. Bosher (1) found that potatoes grown in fields previously planted with bulbous iris in Vancouver, British Columbia, were infested with *Ditylenchus*, and the lesions on these potatoes were confirmed by Dr. A. D. Baker, Chairman, Nematology Section, Entomology Research Institute, to be typical of that of *D. destructor*.

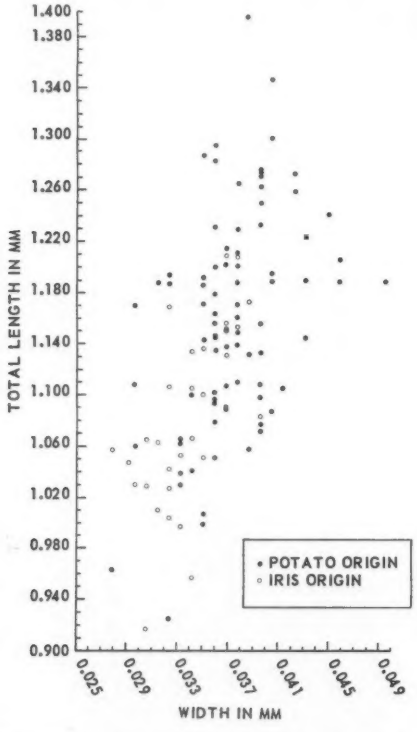


FIG. 3. Relation between width and total length.

Recently, Jensen (3) also expressed the opinion that *D. destructor* infested bulbous iris. In the same article he reported that *D. destructor* was found in dahlia from the same locality, Portland, Oregon.

The ratios of *a*, *b*, and *c* of de Man (2), and the practice of indicating the vulva position in terms of percentage (*V*%) from the anterior end, have been widely used in nematode taxonomy ever since his time. To examine the validity and usefulness of these ratios, statistical analyses have been made on the data for female nematodes of *D. destructor* from potato and iris.

As may be seen from Figs. 4 and 5, the relation between body length and oesophagus length, and between body length and tail length, was not clearly defined within either group of nematodes, and the correlation between these pairs of variables was not significant ($P>0.05$). The use of the ratios *b* and *c* is thus unsupported by the data.

Figure 3 shows the width plotted against the total length, with a clearer relationship between the two variables, and analysis showed that the correlation was highly significant ($P<0.001$). However, a straight regression line relating the two measurements did not pass through the origin, so that *a* is also an unsuitable ratio, on the basis of the present data.

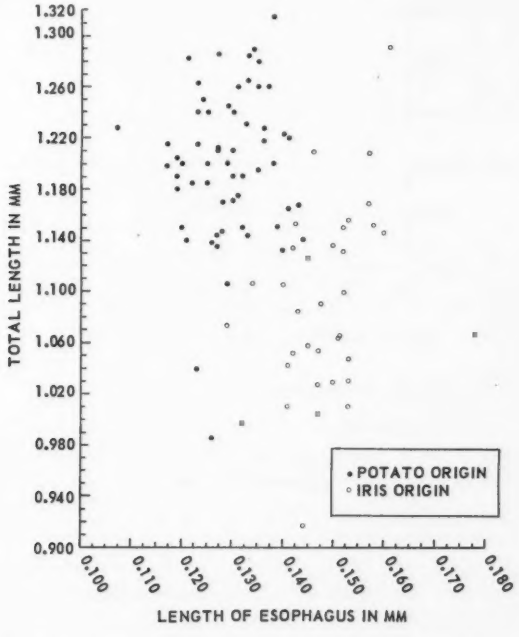


FIG. 4. Relation between length of oesophagus and total length.

As in the case of females, regression of width on length in males was highly significant, with the regression line not passing through the origin. However, unlike the results for females, the regression of length of tail on the total length of body in males was highly significant ($P<0.001$), with the regression line through the origin adequately describing the results (graph not shown). This indicates that the taxonomic value of $c$ in males is supported by the data.

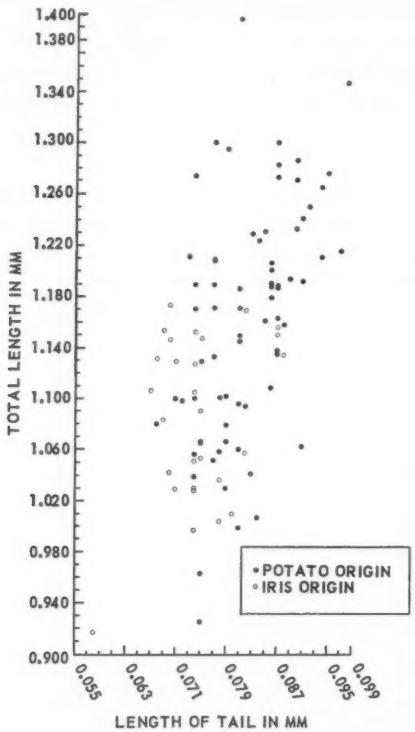


FIG. 5. Relation between length of tail and total length.

There was found to be a highly significant correlation ($P<0.001$) between total length and distance of vulva from the anterior end, and the regression line did not deviate significantly from the origin (Fig. 6). On this basis, therefore, the ratio $V$ has some support from the data. However, the location of the vulva, in this case, is near the posterior end, about 80% of the total length of the nematode, and this may have some bearing on the apparently close relationship. Other data from genera with equatorial and pre-equatorial vulva would be necessary to determine the usefulness of $V$ as a discriminatory function.

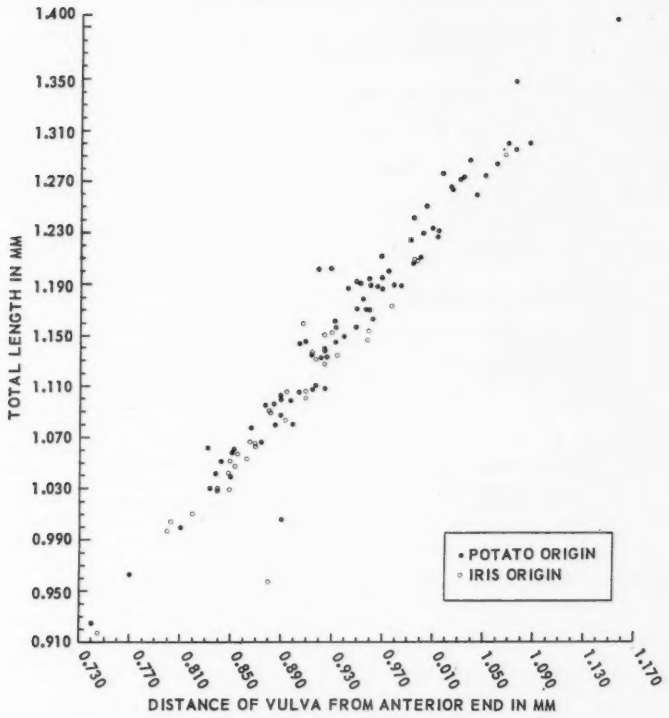


FIG. 6. Relation between length of vulva from anterior end and total length.

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The writer wishes to express her gratitude to Mr. J. E. Bosher, Plant Pathology Laboratory, Saanichton, British Columbia, who kindly sent us the infested iris bulbs. Thanks are also due to Mrs. P. M. Morse, Statistical Research Service, for her help in the statistical analysis.

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